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REVIEW PAPER

Nanoparticle-based stationary phases for pharmaceutical and biomolecule separations

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ABSTRACT

Due to challenges in providing a multi-purpose chromatographic column, columns are modified to acquire specific chemical characteristics for hydrophobic, hydrophilic, ionic or other interactions. Particular properties of nanoparticles such as large specific surface, high pore volume, narrow particle size and pore size distribution, and excellent stability make them perfect supports for electrochromatography and chromatographic applications, which enhance the retention and separation efficiency. On the other hand, because of disadvantages of silica stationary phases, it is noteworthy to design new stationary phases for the separation process. In addition, the applications of nanoparticles in separation process give rise to reach a suitable mass transfer, which is significant in chromatographic science. The present literature survey focuses on various nanoparticle-based stationary phases for the analysis of biomolecules and chiral drugs using capillary electrochromatography and liquid chromatography techniques, which provide rapid and efficient separations, short run time, high enantioseparation, and less consumption of expensive stationary phases.

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INTRODUCTION

Nanoparticles (NPs) with the particle size of 1 up to 100 nm have attracted a great attraction in sciences of chemistry, physics, materials, and medicine because of their special properties. Large surface area of NPs results in an appropriate mass transfer and increasing separation efficiency [1, 2] which are important factors in binding several functional groups onto the surface [3, 4]. Despite the benefits of NPs, their utilization as a stationary phase (SP) in electrophoretic and chromatographic separations is still a challange. Different NPs such as metal oxide NPs, magnetic nanoparticles (MNPs), gold nanoparticles (GNPs), and carbon nanotubes (CNTs) have been suggested for SPs. NPs are also used as SP for enantioseparation. However, proteins [5, 6], polysaccharides [7, 8], cyclodextrins and their derivatives [9, 10], macrocyclic antibiotic [11], chiral crown ethers [12], neutral donor-acceptor selectors [13], chiral ion-exchangers [14], and ligand-exchange selectors [15] have been developed for the enantioresolution of capillary electrochromatography (CEC) and high performance liquid chromatography (HPLC). Among them, with high ratio of surface-to-volume, high chemical stability, special physical and chemical properties, NPs provide improving separation performance. Therefore, they have been generally used in chemistry, physics, materials, medicine, and optics.

Pseudostationary phases (PSPs), monolithic columns, and silica columns have been modified using various NPs. PSPs are considered as

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interaction phases, which are moving with or contrary the mobile phase. In PSP, no column filling or frits are used, the PSP is continuously changed, and a new column is used for each new determination. The mentioned properties give rise to achieve a rapid equilibration and no carry-over effects [16]. Ionic micelles [17], microemulsions [18], cyclodextrins [19], dendrimers [20] and NPs [21] have been recommended as the packing of PSPs.

Monolithic capillary columns have shown a great attraction in enantioseparation process owing to thier high permeability, quick mass transfer, and easy preparation [22-26]. Copolymerization of functional monomers, the chemical amendments of monolithic surface, the incorporation of NPs into the polymeric scaffold, and grafting polymerization have been applied to modify surface chemistries of monolithic columns [1, 27-31]. Modification of monolithic columns with NPs provides a large surface-to-volume ratio leading to a specific chemistry. Some researchers have modified the monolithic columns with NPs because of unique porous structure, excellent hydrodynamic characteristics, and the ease of employing the high flow rates [32-36]. NPs can be loaded into the polymer monolith supports via polymerization of their dispersion in monomers and porogens [37, 38] or are joined to the pore surface of desired monoliths [39, 40].

To prepare a monolithic column with a desired permeability and a favorable surface area, a porogen is used to form preferred morphologies [41]. Cyclohexanol with 1-dodecanol [42] and a mixture of dioxane and water were applied as the porogens [43]. Monolithic columns including rigid organic polymer appeared two decades ago [44, 45] and have been recognized as a suitable SP for rapid liquid chromatography (LC) [46, 47]. They have a little resistance to flow which allows fast separation of proteins and other large molecules [1, 44, 47-50]. Aydoğan and Rassi suggested two chief benefits for monolithic columns: (i) easy preparation using in situ polymerization process in columns and (ii) providing excellent flow [35]. In the current review, monolithic columns were modified using mesoporous silica NP [32], methacryloyl fumed silica NP (MFSNP) [35], GNP [51, 52], hydroxyapatite NP [53], fumed silica nanoparticles (FSNPs) (36), poly(divinylbenzene) (polyDVB) [34, 43], poly(ethylene glycol dimethacrylate) (polyEDMA) [34, 43], and Ag-NPs [42].

Immobilization of NPs within the capillary columns leads to a stable SP [32]. Regarding characteristics of NPs, it is noteworthy to describe some applied NPs as SPs in separation techniques. Among different kinds of NPs, MNPs are a good subject owing to their superior properties such as good dispersion, outstanding biocompatibility, high field irreversibility, easy preparation, large surface area, and physicochemical stability [54, 55]. MNPs were extensively applied in the field of biotechnology such as protein selective separation [55-59], nucleic acid extraction [60], bacteria trapping [59, 61-63], biomedicine [54] and biosensing [63]. However, as indicated by the literature, a few MNPs have been utilized as the SP of CEC [64]. MNPs can be modified using biological molecules to definitely react with the biological compounds of interest [65]. Additionally, an external magnetic field can change their magnetic effects [65, 66]. MNPs are generally based on magnetic elements; for example, iron, nickel, cobalt and their oxides such as magnetite (Fe_3O_4), maghemite (γ-Fe_2O_3), cobalt ferrite (Fe_2CoO_4), and chromium dioxide (CrO_2). In the current review, Wang et al. [64], Liang et al. [67], Yang et al. [68], Sun et al. [69], and Liu et al. [70] used MNPs for the separation of biomolecules and drugs.

Gold nanoparticles (GNPs) as other types of NPs will be discussed because of their compatibility with biomolecules. There is a high affinity between gold and thiol groups. Therefore, the surface of GNPs can be changed. Simple and cheap preparation, easy formation of active materials with biological compounds, tunable particle size with distribution of narrow size make GNPs very attractive in nanoscience [71]. Usually, applied GNPs in separation fields are synthesized in liquid phase using chemical reduction of gold(III) existing in the form of $HAuCl_4$ or $AuCl_4^-$ [72]. Chemical concentration, surfactants, pH, and temperature can affect the size and shape of gold particles [72]. In the present review, the attachment of GNPs to the surface of capillary columns of CEC, HPLC columns and TLC plates have been reported [73-75]. To the best of our knowledge, 47% of NP-based SPs contain GNPs, which is higher than the contribution of other NPs.

Since the discovery of carbon nanotubes (CNTs) in 1991, they have been generally known as ideal nanomaterials in nanoelectronic devices, catalyst supports, biosensors and hydrogen storage with high strength, unique topological and electronic

properties [37, 76-80]. CNTs exist as single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs), which are formed by van der Waals interactions to create a dense, robust and high hydrophobic structure. SWCNT includes a sheet of graphene (G) turned around a cylinder with a typical diameter of 1 nm, but MWCNT as a concentric cylinder has shown an interlayer spacing of 3.4 Å and a diameter usually in the range of 10-20 nm [37]. Dispersion of CNTs in aqueous media and in some organic solvents is challenging [81], because dispersion of CNTs in aqueous solutions causes re-agglomeration, which can produce an unstable baseline, irreproducible retention times and column obstruction [82]. To overcome these limitations, mechanical or chemical methods are employed. In mechanical procedures, sonication of CNTs suspension is done to acquire a temporary dispersion. Physical preparation (non-covalent) can be performed via addition of a surfactant after sonication. However, chemical treatment (covalent) can be accomplished by surface modification of CNTs using acids, sonication and high temperature [83]. Since the application of CNT in chromatography is limited, due to insolubility in the recognized solvents [37], among the studied reports, SWCNTs and MWCNTs have been used as a SP of CEC [81, 84].

The packed atoms of carbon in a hexagonal crystalline array and as a sheet of two dimensional arrangement generate G material [85], which is considered the fundamental form of all graphitic structures [86, 87]. The applications of G for the extraction of benzenoid compounds have attracted considerable attention due to the high delocalized π-electron system of G, enabling the π-stacking interaction with the benzene molecules [88-90]. Recently, G, known as a mono-layer of graphite, has become the subject of intense research because of its high thermal and electronic conductivity, great mechanical characteristic, and large surface area [67]. Hence, many investigations have reported numerous applications of G in several fields such as nanocomposites [91], nanoelectronics [92], supercapacitors [93], ultrasensitive sensors and batteries [94]. The lamellar form of G and the absence of functional groups in its structure lead to its unsuccessful utilization as SP in LC technique [95]. Accordingly, in the current review, modification of G was carried out using MNPs for separation of several amino acids, alkylbenzenes, polycyclic aromatic hydrocarbons, amines, and phenolic compounds [67]. The chemically amended sheet of G gives rise to the formation of graphene oxide (GO) [96]. Unlike hydrofobic and nonpolar structure of G, GO structure contains a lot of oxygen functional groups such as epoxide, hydroxy, and carboxylic acid [97, 98]. This property makes GO a promosing support for its interaction with amino groups of biomolecules and amino acids. Consequently, some investigations used GO NPs to separate several proteins, amino acids, alkylbenzenes, polycyclic aromatic hydrocarbons, amines, and phenolic compounds [67, 95, 96].

Silica nanoparticles have received a great deal of attention because they show poor cytotoxicity, superior chemical stability, simple chemical modification, and mass transfer facilitation owing to their large surface-area. These properties give a platform for the attachment of numerous compounds for separation [16, 99, 100]. Therefore, the large number of investigations has been developed to modify columns using silica NPs. Mesoporous SiO_2 NPs (MSNs) with 2D network of honeycomb-like porous structure include hundreds of vacant channels [101]. Large surface area, pore volume (>1 cm^3g^{-1}), stable mesopore structure, controllable pore diameter, two functional surfaces, and adjustable morphology have been described as the structural properties of MSNs [102].

Considering the significant applications of NPs and nanostructured materials, the current review describes various NPs as SPs of CEC, HPLC, and thin layer chromatography (TLC) techniques by focusing on separation of biomolecules and chiral drugs.

NANOPARTICLES AND CAPILLARY ELECTROCHROMATOGRAPHY

As a separation technique, CE is used to separate ionic components and is based on the difference in electrophoretic mobility of charged analytes under applying an electrical field [103]. Electrophoretic procedures are known as the effective methods for the analysis of peptides and proteins because applying a potential difference affects the separation process based on the ratio of charge-to-mass [104]. However, there are some drawbacks with CE; for instance, the incapability to separate nonionic compounds and analytes with the same ratio of size-to-charge. To resolve these limitations, CEC, which combines the benefits of high selectivity of HPLC and high efficiency of CE, is suggested [105-107]. Several types of columns comprising packed,

monolithic, and open tubular (OT) columns are generally used in CEC technique. Among them, OT columns are of interest for the detection of complex compounds due to lack of bubble formation, easy preparation, and simple instrumental management [64]. In order to overcome the defects of open-tubular capillary electrochromatography (OT-CEC) such as inadequate amount of SP coating, low phase ratio and poor sample capacity of OT columns, new methods including coatings using polymer, layers of porous silica, etching, and sol-gel techniques have been developed, so that the surface area and the interaction between the compounds and the coated SP are improved [108, 109]. A brief description of the mentioned methods will be described here. In etching method, a novel chemical material, which influences both the electroosmotic flow (EOF) and the adsorption properties of the capillary, is formed at the inner wall of the capillary column. This process gives rise to achieve a large surface for the inner wall of the capillary column [110, 111].

In 1987, sol-gel technology, which is used as a separation column in LC, was applied to fill monolithic ceramic support of capillary with small diameter, [112]. The sol-gel method provides some advantages such as: (i) favorable homogeneity and high purity from raw materials; (ii) possibility to control the size, shape and properties of particles; (iii) possibility to create the material structure with new property via appropriate selection of sol-gel components; and (iv) high stability of SP and performance in chromatographic analyses [113]. To prepare SPs based on the sol-gel process, the chemical reagents are required: (1) one or more precursors, which is commonly a metal alkoxide [$M(OR)_x$] [114], (2) a solvent for the dispersion of the precursor(s), (3) an acidic, basic or a fluoride catalyst [115-118], and (4) water [41].

Separation of chiral amino acids and drugs are continuously growing because L-enantiomers of amino acids show appropriate activity, whereas D-enantiomers are inactive molecules with different properties and toxic effects [9, 10]. On the other hand, the pharmacodynamic and pharmacokinetic of particular enantiomer influence the metabolism of chiral drugs, which can affect the progress of new drugs [13]. In separation of enantiomer, CEC compounds is a suitable technique. To do this, the internal wall of capillary column is coated with the chiral selector which resolves the problems of packed columns. Due to the specific properties of NPs in separation process, they are good for supports to load chiral selectors in OT-CEC [119]. In the current report, enantioseparation of chlorpheniramine, zopiclone, tropicamide [52] and enantioseparation of amino acids [75, 120] were performed using CEC.

So far, according to Qu et al., two chief methods have been applied to modify the capillary column using NPs [121]: (i) coating the pre-derivatized fused silica capillary with NPs via chemical bonding [122-124] and (ii) immobilization of one NP layer onto the capillary wall by a chemical reaction at high temperatures [125-127]. In the following sections we report the use of NPs as SP of CE technique for separation of biomolecules and drugs.

Biomolecules

To further improve the separation process, functionalization and augmentation of the surface's charge density of modified CNTs is necessary. Thus, loading SWNT into an organic polymer monolith containing vinylbenzyl chloride (VBC) and ethylene dimethacrylate (EDMA) provided a monolithic SP for micro-HPLC and CEC, which resulted in improving the peak efficiency and increasing the chromatographic retention of small neutral molecules in RP-HPLC due to the hydrophobic interaction. Separation of a peptide mixture comprising Gly-Tyr, Val-Tyr-Val, methionine enkephalin, leucine enkephalin, and angiotensin II was carried out by Li et al. using VBC-EDMA-SWNT SP [37]. MWCNTs provide a larger surface area than that of SWCNTs; therefore, the functionalization of the large surface area of MWCNTs can enhance the surface charge density because of high coverage of functional groups on the multiple layers of G [84]. Hence, modification of MWCNTs using carboxylated (COOH), hydroxylated (OH), and sulfonated (SO_3H) groups coated with sodium dodecylsulfate (SDS) as PSPs in electrokinetic capillary chromatography (EKC) technique was performed by Alharthi and Rassi to study the retention of some molecules [81]. For the first time, MWCNTs-OH and MWCNTs-SO_3H as PSPs were introduced in this study. Comparison of functionalized MWCNTs with unmodified type showed better results for electrophoretic force. In addition, it was discovered that MWCNT-COOH is a good choice for the separation of nucleic acid bases and nucleosides, owing to interactions of hydrophobic, hydrogen

bond and π-π stacking between the analytes and the MWCNT-COOH [82, 128]. However, neutral solutes such as alkylbenzenes (ABs), phenyl alkyl alcohols (PAAs) and alanine derivatives in neutral types were not separated by MWCNT-COOH and MWCNTs-SO_3H due to the insufficient surface charge density of the modified MWCNTs for AB and PAA compounds. Therefore, the SDS coated unmodified and functionalized MWCNTs to make better separation of neutral components. Also, based on the Cao et al.'s studies, application of SDS-coated SWCNTs gives a more efficient resolution than unmodified carboxylated SWCNTs [129]. Moreover, in another investigation, Alharthi and Rassi studied the retention behaviors of small and large solutes in nanoparticle capillary electrokinetic chromatography (NPEKC) [84]. MWCNTs-COOH, MWCNTs-SO_3H, and MWCNTs-OH were coated using SDS as a good PSP for the separation of herbicides, barbiturates, dansyl-DL-amino acids, dipeptides and proteins by NPEKC. In addition, myoglobin, cytochrome C, and lysozyme were investigated, and high resolution, selectivity and efficiency were obtained for all of compounds.

Modified GNPs with poly(ethylene oxide) (PEO) were used as a filling of CE column by Huang's team to resolve the problems of long-stranded DNA separation using CE, which resulted in a quick and highly efficient separation [130]. The molecules of PEO bond to the surfaces of GNPs using hydrophobic groups, improving the accessibility of the hydrophilic groups of PEO to interact with the polar compounds [131, 132]. Similarly, in another study performed by Tseng et al., GNPs were used for the separation of long DNA molecules by CEC under the application of hydrodynamic and electrokinetic forces [133]. Compared to Huang et al.'s results [130], the proposed method provided a quick separation, high resolution, great reproducibility, simple preparation, and poor unfavorable peaks.

Analysis of acidic and basic proteins using the modified didodecyldimethylammonium bromide (DDAB) with GNPs as PSP resulted in a superior separation efficiency, excellent reproducibility, and high reversed EOF [134]. In this study, to acquire better interactions with proteins, the GNPs were modified by PEO through noncovalent bonding interactions to produce composites of GNPs/polymer (GNPPs). It was found an excellent peak efficiency for basic proteins. Therefore, it is possible to use the established method for the analysis of highly basic, macrophage proteins in mammalian cells [135], and myelin basic protein in cerebral spinal fluid [136].

Modification of GNPs by thiolated β-cyclodextrin (β-CD) as a PSP in CEC was performed by Yang and coworkers to analyze four couples of dinitrophenyl-labeled amino acid enantiomers namely, DL-Val, Leu, Glu, and Asp; and three couples of drug enantiomers including RS-chlorpheniramine, zopiclone and carvedilol [71]. A good theoretical plate number (up to 2.4×10^5 per meter) and separation resolution (up to 4.7) were achieved by GNPs as a chiral selector. Solubility of thiolated β-CD-GNP and its stability in both acidic and basic conditions give rise to an appropriate PSP for enantioseparation. To modify the fused silica capillaries of CEC, Qu et al. coated n-octadecanethiol onto the surface of GNPs via layer-by-layer (LBL) method for the separation of polycyclic aromatic hydrocarbons (PAHs), basic and acidic proteins [121]. To do so, positively charged GNPs were fabricated and immobilized onto the capillary wall modified via two layers of polyelectrolyte. The new modification not only was robust with an application of more than 810 analyses, but also demonstrated high chemical stability versus NaOH (0.01 M), HCI (0.01 M), and concentrations of electrolyte up to 70 mM. LBL method is a good subject to avoid the absorption of basic molecules onto the capillary column and has drawn considerable attention in OT-CEC [137-139]. In this method, a polyelectrolyte was used to modify the inner surface of fused silica capillary [140, 141].

Conjugation of bovine serum albumin (BSA)-GNPs as a chiral stationary phase (CSP) of CEC was performed for enantioseparation of amino acid derivatives with phenylisothiocyanate (PITC), which resulted in an analysis time of 18 min [75]. Enantiomers of tryptophan, tyrosine, leucine, phenylalanine, serine, β-phenylalanine, aspartic acid, alanine, threonine, and arginine were evaluated by CEC. The sol-gel method was applied to prepare the bare monolithic silica column and has been modified chemically with 3-mercaptopropyltrimethoxysilane to provide thiol groups, immobilization of GNPs through the fabrication of an Au-S bond, and modification with BSA as the chiral selector.

For excellent separations of synthetic peptides mixture, a modified fused silica capillary with GNPs was applied by OT-CEC technique [104].

It was proved that the obtained capillary can be reused almost 900 times and separation of peptides depends on the interaction between peptides and GNPs which expressively change the EOF.

OT-CEC using GNPs SP was used to analyze glycated proteins, BSA, and human transferrin [72]. The inner wall of the fused silica capillary was coated by bare GNPs. GNPs were prepared according to the reduction of Au(III) via trisodium citrate dehydrate. Sodium phosphate buffer 100 mmol/L at pH 2.5, separation voltage 10 kV per 47-cm long, inside diameter capillary of 50 μm, and temperature of 25 °C were described as the best separation of peptides.

Krenkova et al. used commercial hydroxyapatite NPs for the protein separation (ovalbumin, myoglobin, lysozyme, and cytochrome C) and selective enrichment of phosphopeptides via the monolithic capillary columns of CEC [53]. Hydroxyapatite as a SP of chromatography is a crystalline form of calcium phosphate with the structural formula of $Ca_{10}(PO_4)_6(OH)_2$ that was established by Tiselius [142]. Hydroxyapatite NPs as the commercial form with dimensions of 50 nm are very small to be applied directly as a SP. However, immobilization of hydroxyapatite NPs onto an ideal porous was suggested by Krenkova et al. to introduce SPs of capillary columns based on the hydroxyapatite. The mass transfer achieved was fast, which allows hydroxyapatite NPs to be used in in-line control of biotechnology procedures wherever throughput is serious.

For the enantiomeric separation of derivatives of amino acids with 9-fluorenylmethoxycarbonylchloride, Domínguez-Vega et al. synthesized cellulose tris(3-chloro-4-methylphenylcarbamate), recognized as Sepapak-2 or Lux Cellulose-2, as a polysaccharide-based CSP to study amino acids of Ser, Thr, Asn, Gln, Cys, Pro, Ala, Lle, Leu, Allo, Met, Phe, Trap, Val, Asp, Glu, Pipe, Pyro, Lys, Cit, Orn, Hys, and Arg [120]. The separation of 19 out of 23 enantiomeric amino acids by nano-LC represented high chiral detection for this novel CSP. A comparison of nano-LC and CEC under the similar conditions gives superior peak efficiencies and resolution by using CEC experiments, which made possible the chiral discrimination of 20 out of 23 amino acids tested. Compatibility with the organic and aqueous solvents, enantioselectivity properties towards various molecules, the easy accessibility of natural sources, and the possibility of high incorporation of a chiral selector onto an inert carrier were mentioned as CSPs properties of polysaccharide-type [143]. Short run times of analysis for derivatives of essentially uncharged amino acid at low pHs were performed by using aminopropylized silica as the support of CSP [120]. Usually, modification of the silica surface with coating or covalent-bonding of polysaccharide phenyl esters or phenylcarbamates leads to obtain polysaccharide-type CSPs [144].

In another investigation, Qu et al. evaluated nanosheets of GO and G for OT-CEC to assay ovalbumin, ovotransferrin, ovomucoid, ovoflavoprotein, lysozyme, and avidin in chicken egg white [96]. 3-aminopropyldiethoxymethyl silane as a coupling agent was used to immobilize nanosheets of GO onto capillary column. Coating of G onto the column was done via hydrazine reduction of GO. Investigations of Qu and coworkers showed a pH-dependent EOF from anode to cathode in the pH of 3-9 for G-column and a constant EOF for GO-column. Finally, GO-column showed a good separation for the neutral analytes, however the poor separation was achieved for G-column.

To improve the chromatographic separation of myoglobin, ribonuclease A, lysozyme and α-chymotrypsinogen A, Arrua et al. prepared cryopolymers using embedded polyDVB and polyEDMA by mini-emulsion polymerization [43]. The obtained cryopolymers with an open porous structure and considerable specific surface area are interesting compounds for the separation of biomolecules by hydrophobic interaction chromatography (HIC). The polymerization mixture was comprised of poly(ethyleneglycol) diacrylate (PEGDA) as the single monomer, a combination of dioxane and water as the porogen, N,N,N',N'-tetramethylethylenediamine (TEMED) and ammonium persulfate as the initiator system. Although the peak capacity and resolution factor were lower than those described for conventional columns of methacrylate monolithic, the use of this polymerization method gives rise to a polymeric arrangement with a more open porous structure and higher permeability than conventional polymer monoliths. In another investigation made by Arrua et al., the incorporation of charged NPs such as NR_4^+ and SO_3^- onto the polymer surface was carried out using direct addition of their suspensions for the polymerisation mixture to analyze myoglobin, ribonuclease A, lysozyme and α-chymotrypsinogen A [34]. Compared with the

previous study performed by Arrua et al. [43], this ionic modification allows the application of columns in ion-exchange chromatography. The results indicated that the modification of monolithic columns using the direct addition of NPs is a suitable choice to functionalize monolithic polymers without changing the polymeric scaffold. Arrua et al., in two different studies [34, 43], showed that although addition of neutral NPs enhanced the chromatographic separation of biomolecules, a lot of NPs were buried within the polymeric scaffold and, hence, the potential advantages of this methodology could not be exploited completely. In emulsion polymerization, dispersion of the monomer is performed in an aqueous solution of surfactant with the critical micelle concentration (CMC). In the following, an initiator agent, commonly a water-soluble compound, is used to begin the polymerization process [145]. Entry of particles into the micelle, growing oligomers precipitation in the aqueous phase, and entry of particles into droplets of monomer caused to form the polymer particles [145]. The expression of mini-emulsion was used to explain the submicron droplets of oil in water dispersions that are stable for a long time from several hours to months [146].

Immobilization of BSA onto GO-magnetic nanocomposites (GO-Fe_3O_4-BSA) as SP of OT-CEC was reported for the effective enantioseparation of tryptophan, threonine, and propranolol in less than 80 s with resolution factors of 1.22, 1.9, and 2.1, respectively [67]. The new NPs of GO-Fe_3O_4-BSA has magnetism properties of Fe_3O_4 NPs, larger surface, and good biocompatibility of G, leading to immobilize further biomolecules and well sustain their biological activity. GO-Fe_3O_4-BSA conjugation was packed into microchannels of poly(dimethylsiloxane) using the magnets, which facilitates the immobilization of protein and gives a pattern for high efficiency screening of enantiomer compounds.

Modification of MNPs with carboxyl group was applied for separation of amino acids, dipeptides and proteins using OT-CEC [64]. MNPs of Fe_3O_4-COOH, made through solvothermal reduction process, were loaded on the surface of positively charged poly(diallydimethylammonium chloride) (PDDA) via electrostatic self-assembly to evaluate Trp, Tyr, and Phe as amino acid, Gly-Trp, Gly-Tyr, and Gly-Phe as dipeptide, ConA, α-Lac, β-Lg, and BSA as protein. Separation of egg white as a real sample via OT column coated with PDDA@Fe_3O_4-COOH MNPs revealed a superior resolution for proteins compared with a bare fused-silica capillary. PDDA as a water-soluble cationic polyelectrolyte has been commonly used to make semipermanent coating in OT-CEC through electrostatic interaction [147-150]. PDDA with quaternary ammonium groups and positive charges make an innovative SP via electrostatic self-assembly with different NPs [64]. Furthermore, Yang et al. modified MNPs using β-CD (MNP-β-CD) and ionic liquid of mono-6-deoxy-6-(1-methylimidazolium)-β-cyclodextrin tosylate (MNP-β-CD-IL) for enantioseparation of dansylated forms of alanine, leucine, lsoleucine, valine, methionine, and glutamic acid using CEC technique [68]. In comparison with uncoated capillary, suitable reproducibility, stability, and high enantioseparation resolution were observed. The morphology of MNPs was studied by tunneling electron microscopy technique, through which the average diameter of 8-12 nm was obtained. Also, based on the scanning electron microscopy analysis, the coating thicknesses of MNP-β-CD and MNP-β-CD-IL were nearly 5-15 μm and 5-20 μm, respectively.

Another modification of MNPs was performed via GNP for separation of neutral compounds, isomers, ovalbumin, ovotransferrin, ovomucoid, lysozyme, and avidin [70]. The bifunctional dumbbell-like Janus GNP-MNP was fabricated using hydrothermal synthesis method. To do this, GNP with particle size of 5 nm was conjugated to MNPs with particle size of 11 nm. Also, it was indicated that modified capillary with MNPs and GNP-MNPs showed lower EOF than that of the uncoated silica column, and that modified capillary via MNPs had no enough resolution for thiourea, naphthalene, and biphenyl.

In another study, modification of silica NPs was done using 3-aminopropyltrimethoxysilane and N^1-(3-trimethoxysilylpropyl)diethylenetriamine based on the hydrophilic interaction, resulted in monoamine- and triamine-bonded silica NPs with a particle size of 20 nm as PSP of CEC to separate oligosaccharide derivatives. These amendments produced quick EOF of 2.59×10^{-4} $cm^2v^{-1}s^{-1}$ in the direction of the anode in an electrical field, which allows a rapid analysis [21]. Also, isomers of benzenediol, catechol, resorcinol, hydroquinone, uracil, adenine, cytosine, and guanine were separated via monoamine-bonded silica NPs. Separation of oligosaccharide derivatives is based on the polymerization degree (DP). Monoamine-

bonded silica facilitates the separation of the saccharides with a DP of 1 up to 17, whereas the larger oligosaccharides co-migrated. Hence, to resolve the oligosaccharides separation, the triamine-bonded silica is a suitable selection.

For the first time, lysozyme, cytochrome C, and α-chymotrypsinogen A as the protein and PAH were separated using NPs of fibrous mesoporous silica (fSiO$_2$) grafted with poly(2-(dimethylamino) ethyl methacrylate (PDMAEMA) as a SP for OT-CEC [99]. PDMAEMA is a polymer with positive charge, which its charges change against to pH of solution [151] and can be easily adsorbed to a silica capillary inner wall via strong interaction of electrostatic [99]. Moreover, functionalization of SP based on PDMAEMA polymer by phenylalanine [152] or polyethyleneimine (PEI) [153, 154] have been wildly explored for OT-CEC to increase phase ratio. Separation of egg white proteins such as ovalbumin, ovotransferrin, ovomucoid, lysozyme, and other proteins were investigated, which could not be easily separated on the bare capillary owing to interaction of the analytes with the wall of column. The relative standard deviations of run-to-run and day-to-day for EOF and retention time of analytes showed a reproducible method for the preparation of P-f SiO$_2$@C$_{18}$ column.

Pharmaceuticals

Owing to stereoselectivity of interaction between drug and target, only one of the two enantiomers can provide the desired pharmacological effects, while the other one may exhibit less activity, minor effectiveness, poisonousness, and unwanted side effects. Hence, chirality is a crucial subject in many fields such as environmental, food, pharmaceutical, and especially the science of human health [155]. In this regard, among the proposed processes for analysis of enantiomers, proteins have been of particular interest because of various binding strengths with enantiomers. So recently, some investigations have been done to improve chiral separations using proteins. In this regard, discrimination of ephedrine and norephedrine isomers was investigated by BSA-GNP as a chiral SP in chip-based enantioselective OT-CEC [156], providing high speed, integration of sample preparation and introduction as on-line, controllable sample injection, high efficiency and low amount of sample [157-159]. A microchip is considered as an analytical device which all the steps of a measurement including preparation, separation, and detection of analytes are done on a small footprint [160-162].

Modification of GNPs with thiolated β-CD as a SP of OT-CEC was applied for enantioseparation of zopiclone, tropicamide and chlorpheniramine [119]. PDDA followed by self-adsorption of negatively charged CD-GNPs was used to fabricate enantioselective OT capillary column. In this report, Li's team demonstrated that the column shows good repeatability for enantioseparation, and enantioselectivity of the column can preserve for more than 1 month provided that the column was kept in solution of CD-GNPs at 4 °C. Limit of detection (LOD) was reported as 8.8 for zopiclone, 6.3 for tropicamide and 5.4 μg/mL for chlorpheniramine. In another research, Li et al. amended GNPs with β-CD (β-CD-GNP) through covalent interaction as the SP of CEC for enantioseparation of chlorpheniramine, zopiclone, and tropicamide [52]. The new column with theoretical plate numbers of 1.28×10^5 displayed stable EOF over pH ranges from 4.6-9.7. Similar analysis using β-CD-GNP was done for enantioseparation of chlorphenamine, brompheniramine, pheniramine, and zopiclone [163]. The sol-gel strategy was used to prepare the chiral OT-CEC column via β-CD. It was indicated that the GNPs as the SP improved the phase ratio of column, which resulted in improving the selectivity of chiral column. Another investigation reported carboxymethyl-β-CD conjugated GNPs as SP of two pairs of α-tetralones derivatives enantiomers and enantiomers of tramadol hydrochloride and zopiclone [164]. LBL self-assembly method was applied to fabricate the OT-CEC column. Compared to the bare capillary, the suitable stability for EOF was achieved under the working pH range.

With regards to favorable properties of GNPs, the racemic β-blockers of propranolol, esmolol, bisoprolol and sotalol were analyzed using GNPs coated capillary [165]. L-cysteine and carboxymethyl-β-CD as chiral reagents were used to modify GNPs. Moreover, tetramethylammonium lactobionate was selected as a chiral marker. In this condition, based on the scanning electron microscopy analysis, L-cysteine provided insignificant change to the column, while, modification using carboxymethyl-β-CD created a bit bigger surface.

Enantioseparation of nefopam, chlortrimeton, azelastine, clenbuterol, ritodrine, esmolol,

amlodopine, citalopram, propranolol, metoprolol, bisoprolol, salmeterol, atenolol, labetalol, and sotalol in less than ten minutes on pepsin-based poly(GMA-EDMA-NH_2-MSN) monolithic column of CEC was done by Xu et al. [32]. Pepsin, as a type of aspartic protease, is usually utilized as a bioreactor and less attention has been paid to its enantioresolution characteristics [32]. NH_2-MSN gets the required stability for enantioseparation purposes and functional groups to immobilize pepsin, which can augment the chiral resolution capacity.

Different literatures presented the benefits of porous polymeric materials as an innovative SP for selectivity improvement and chromatographic performances [166-168]. Grzywiński et al. changed the monolithic column in fused-silica capillaries with cystamine dihydrochloride, subsequently Ag-NPs immobilization, which was functionalized via cholesterol cysteamine to separate ribonuclease A, cytochrome C, and myoglobin using the RP-chromatographic performance [42]. The desired SP did not demonstrate selectivity for low molecular weight compounds owing to the modification in their porosity.

To separate enantiomers of propranolol, ofloxacin, amlodipine, chlortrimeton, tropicamide, and atenolol, Sun et al. modified magnetic microparticles (MMPs) using carboxymethyl-β-CD as SP of OT-CEC column [69]. Tunneling electron microscopy analysis demonstrated the mean diameter of 500 nm and spherical shape for bare MMPs and CD-MMPs. Moreover, compared to the bare capillary column, EOF variation was insignificant, leading to a high reproducibility for migration time and resolution.

The essential information for the mentioned methods is described in Table 1.

NANOPARTICLES AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

To achieve a high-resolution and quantitative separation, chromatographic analyses have been extensively used in separation, materials, environmental, and life sciences. After the introduction of NPs, new achievements have been obtained in chromatographic techniques using NPs as SP [169]. In the following sections we present the application of NPs as SP of HPLC technique for separation of biomolecules and drugs.

Biomolecules

HPLC analysis equipped with GNPs postcolumn was developed by Lu and coworkers for cysteine, cysteinylglycine, homocysteine, glutamylcysteine, and glutathione with the LODs of 2.0 μM, 40, 40, 60 and 80 pmol, respectively in biological fluids. GNPs with the particle size of 12 nm were attached to Triton, Tween, and Brij, as commercially nonionic surfactants, and their interactions with biothiols and biomolecules were studied. Their results indicated a high selectivity for small biothiols and quick mass kinetics for Brij 35. Easy preparation, high stability in aqueous media over an extensive pH range at room temperature, and high selectivity against small biothiols were mentioned as the benefits of GNP colloids [170]. A porous polymer monolithic capillary column of micro-HPLC equipped with mass detector was modified using GNPs, which allowed the selective trapping of peptides including cysteine molecules [51]. The best conditions were found with $HAuCl_4$ of 50 mmol/L and trisodium citrate of 200 mmol/L. A mixture of six peptides, three of them containing a cysteine residue, was tested using the commercial capillary column of C_{18} modified with GNPs, which resulted in retaining of peptides containing cysteine, whereas the other peptides passed [51].

CNPs, with size of 6-18 nm, obtaining from corn stalk soot via refluxing method in nitric acid has been applied as a SP of HPLC to separate five nucleosides, four sulfa compounds and safflower via both of hydrophilic interaction liquid chromatography (HILIC) and *per* aqueous liquid chromatography (PALC) conditions. The produced CNPs were soluble in water owing to the presence of OH and COOH groups and also they showed fluorescence property. Different nucleosides such as thymine, inosine, adenosine, cytosine, and adenine were studied and revealed stronger retention on the CNP column than on amino column, whereas the selected nucleosides were hard to retain on column of C_{18} in HILIC situation. The lowest and the highest retention factors were obtained for thymine and adenine, respectively in both of HILIC and PALC modes [171].

SP of GO@silica modified by octadecylsilane was used to separate alkylbenzenes, polycyclic aromatic hydrocarbons, amines, and phenolic compounds using HPLC [95]. Both of π-π- and hydrophobic interactions between analyte and

Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
Isomers of benzenediol, catechol, resorcinol, hydroquinone, and oligosaccharide derivatives	3-aminopropyltrimethoxysilane @ silica NPs and N^1-(3-trimethoxysilylpropyl)diethylenetriamine @ silica NPs	Silica NPs	Passing 5% aqueous solution of sodium silicate through a column of Dowex 50Wx2 (H^+ form, 80 mL volume)/addition of fractions to aqueous NaOH 10 mM and refluxed for 3 h in an oil bath to get an aqueous solution of 20 nm silica NPs/addition of 3-aminopropyltrimethoxysilane or N^1-(3-triethoxysilylpropyl)diethylenetriamine to solution of the silica NPs/stirrer for 7 h at room temperature/dialysis against MeOH-water with the ratio of 3:7, 5:5, and 8:2 (v/v)/dialysis against MeOH.	CEC	---	[21]
Nefopam, chlortrimeton, azelastine, clenbuterol, ritodrine, esmolol, amlodopine, citalopram, propranolol, metoprolol, bisoprolol, salmeterol, atenolol, labetalol, and sotalol	Pepsin-based poly(GMA-EDMA-NH_2-MSN) monolithic	Nanoparticles of mesoporous silica	The mesoporous silica nanoparticles were modified via amino groups by reaction with APTES, according to the report of (183).	CEC	Preparation of fused-silica capillary with NaOH (1h), HCl (0.5 h)/washing with water and then with MeOH for 10 min/drying with N_2 at 120 °C for 2 h/silanization the pretreated capillary by γ-MAPS (50%, v/v) in MeOH/reaction at 50 °C for an overnight/rinsing the capillary with methanol/drying with N_2/addition of NH_2-MSN to propanol/ vortex for 3 min and ultrasonication for 1 h/addition mixture comprising of 21.96% (v/v) GMA, 7.47% (v/v) EDMA, 8.19% (v/v) 1,4-butanediol, 62.38% (v/v) propanol containing NH_2-MSN and 0.32% (m/v) AIBN into the pre-conditioned fused-silica capillary/reaction at 46 °C for 12 h/washing with methanol and water. **To pepsin immobilization:** Pumping ammonium hydroxide 25% (m/v) into the monolithic column/reaction at 40 °C for 5 h/rinsing with water/flushing with GA 50% (m/v) for 5 h/dissolvation of 2.0 mg mL^{-1} pepsin in 100 mmol L^{-1} ammonium acetate-acetic acid buffer @ pH 4.5 including 1 mg mL^{-1} sodium cyanoborohydride/pushing the obtained solution into the column incessantly for 12 h/pumping 100 mmol L^{-1} ammonium acetate-acetic acid buffer @pH 4.5 for 2 h to remove untreated pepsin.	[32]
Myoglobin, ribonuclease A, lysozyme and α-chymotrypsinogen A	Charged polyDVB and polyEDMA	PolyDVB and polyEDMA		CLC	Preparation of monolithic cryopolymers by free radical polymerisation at temperature of -70 °C with APS-TEMED as a redox initiator system/centrifuge polymerisation mixture at 1500 × g for 10 min/addition of nanoparticles suspension to the clear solution and sonicate for 10 min/deoxygenated with N_2 for 10 min/addition of TEMED to start polymerisation reaction (1 wt% with respect to monomer amount)/filling with the polymerisation mixture by N_2 and sealed at both ends/start polymerisation reaction at -70°C for 6 h/freeze the capillary at -12 °C for 24 h to complete the polymerisation reaction/melt the cryopolymer at room temperature/rinsing with water.	[34]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
Alkylbenzenes, phenol, aniline, 4-methylaniline, N-methylaniline, N-ethylaniline, 3,4-dichloroaniline, lysozyme, ribonuclease A, carbonic anhydrase isozyme II, cytochrome C, α-chymotrypsinogen A, and myoglobin	Poly(GMM-EDMA-MFSNP)-octadecyl	MFSNP	Addition of FSNPs and TMSPM to a solvent mixture of cyclohexanol and dodecanol/vortex for 5 min and sonication for 5 min/heating up to 120 °C for 24 h to obtain MFSNP/treatment of FNSPs with TMSPM/addition of the suspension to the GMM-EDMA solution to synthesize the monolith column.	HPLC	Addition of GMM, EDMA, MFSNP to the solvent comprising of cyclohexanol and dodecanol/addition of AIBN to the polymeric solution/vortex for 5 min, sonication for 5 min, purged with N_2 gas for 5 min at 25 °C/introduction of the final polymer solution into the column/plugging both ends of column/heating the column at 55 °C in a water bath for 15 h/washing the monolithic column with ACN for 30 min/perfuse DODCS (4% w/v) in toluene while the column was kept at 110 °C for 6 h to fabricate desired monolithic column/washing the monolithic column with ACN for 30 min/separation of the top 5 cm column from the 10 cm column/preparation of the blank column with no MFSNPs in the same way.	[35]
Nucleosides, hydroxybenzoic acids and nucleotides	FSNPs @ poly(GMM-EDMA)	FSNPs	Commercial FSNPs.	HPLC	Addition of GMM, EDMA, FSNPs to the cyclohexanol and dodecanol solution/addition of AIBN to the polymeric solution/vortex the mixture for 2 min/sonication for 5 min/purging with N_2 gas for 5 min at 25 °C/introduction of the final polymer solution to column consisting of two sections/plugging both column ends tightly/heating the column at 55 °C in a water bath for 15 h/washing the monolithic column with ACN for 30 min/separation of the top 5 cm column from the 10 cm column/preparation of the blank column with no FSNPs in the same way.	[36]
A peptide mixture	---	SWNT	Growing SWNT for 1 h under 6 atm CO pressure at 800 °C and at the presence of Co-MCM-41/refluxing for 1 h in 2.5 wt % NaOH/addition of SWNT a aqueous mixture of 98% H_2SO_4 and 30% H_2O_2/stirrer for 30 min/sonication for 10 min/dilution SWNT suspension with distilled water and filtering/washing SWNT using 10 mM NaOH and distilled water/dispersion of SWNT mat into 2-propanol/sonication the SWNT suspension in 2-propanol for 15 min before utilize.	μ-HPLC and CEC	Silanization fused-silica capillaries after pretreatment/filling the capillary TMSPM and 0.1% (w/v) DPPH in dry DMF/purging with He for 10 min, and filling the pretreated capillary/sealing both end of the column/placing the column in the oven at 120 °C for 6 h/washing the capillary with DMF, MeOH, and methylene chloride at room temperature/drying with N_2 for 1 h/filling the column with a mixture solution containing VBC, EDMA, 2-propanol containing pretreated SWNT and formamide, and AIBN/sealing and heating the capillary column for 16 h at 75 °C/washing the capillary with MeOH and deionized water/pumping aqueous solution of NaOH through the column/washing the column with deionized water and MeOH/purging with N_2 at room temperature for 24 h to obtain porous monolithic SP.	[37]
Ribonuclease A, cytochrome C, and myoglobin	Ag-NPs @ fused-silica	Ag-NPs	---	CLC	Rinsing the capillary with acetone and dichloromethane/drying under N_2 atmosphere/etching the capillaries using 1 M NaOH (at 100°C for 3 h)/vinylized with 10% solutions of γ-MAPS in toluene (at room temperature for 2 h)/washing the capillaries with toluene and acetone/drying under N_2 atmosphere and left at room temperature overnight before using/polymerizing with functional monomer (24 wt.%), cross-linking monomers (16 wt.%), and cyclohexanol with 1-dodecanol as porogens (60 wt.%) and AIBN (1% w/w) with respect to the monomers)as the initiator/purging with N_2 for 5 min/sonicating for 5 min/ introducing into the vinylized capillaries/sealing both ends of each capillary/keeping in a thermostated water bath at 60°C for 24 h/flushing the monolithic columns with acetonitrile/extraction of monolithic blocks with toluene in the Soxhlet apparatus for 24 h/drying in the vacuum oven at 80°C for at least 2 days/pumping solution of silver colloid to	[42]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
					loop at a flow rate of 2 min^{-1}/refill the loop at volume of 300 µL of the Ag-NPs solution to modify the capillary monolithic column.	
Myoglobin, ribonuclease A, lysozyme, and α-chymotrypsinogen A	Neutral polyDVB and polyEDMA	PolyDVB and polyEDMA	Addition of EDMA or DVB, HD and AIBN to an aqueous mixture including H_2O and SDS/ultracentrifugation in an ice bath at for 6.5 min in 1 min gaps with a 30 s rest/ deoxygenated with N_2 for 15 min/complete the polymerization reaction in an oil bath at 65 ˚C for 16 h/purification using membrane dialysis for 48 h with water changing each 5 h.	CLC	Preparation of monolithic cryopolymers using free-radical polymerization at temperatures of sub-zero by APS/TEMED as redox initiator/addition of PEGDA to a mixture including H_2O, dioxane and APS/centrifuge at 3000 rpm for 10 min/sonication for 10 min/deoxygenated under N_2 for 10 min/addition of TEMED to initiate polymerization reaction/filling the capillary by N_2 pressure/sealing both ends of the capillary and frozen at numerous sub-zero temperatures (-196,-70, and -40 ˚C) for 6 h/storing at temperature of -12 ˚C overnight /melt the cryopolymer at room temperature and rinsed with H_2O for 5 h/for the preparation of cryopolymers with incorporation nanoparticles, a similar method was employed except with the addition of nanoparticles to the polymerization mixture prior to the sonication step.	[43]
Peptide	GNPs @ porous polymer monolithic column	GNPs	Commercial GNP solution	µ-HPLC	Vinylization of the fused-silica capillary/sonication of the mixture of GMA, EDMA, cyclohexanol, 1-dodecanol, and AIBN/purging with N_2 for 10 min/filling the vinylized capillaries with the polymerization mixture/sealing both ends of column/initiate the polymerization in a water bath at 60 °C for 24 h/rinsing the column with ACN (GMA column)/reaction with sodium-hydrogen sulfide as report of (184)/pumping the aqueous solution of cysteamine through the glycidyl methacrylate-based column at a flow rate of 0.5 µL/min for 20 min/rinsing the column with water (SH column)/connection of a T-piece with two inlets to two syringe pumps/delivery of an aqueous solutions of $HAuCl_4$ and sodium borohydride or sodium citrate to T-piece connection/attachment of SH column to the third outlet/rinsing the column with water/storing the column at temperature of 4 °C.	[51]
Chlorphenir amine, zopiclone, and tropicamide	β-CD-GNPs	GNPs	Synthesize CD-GNP using a three-step procedure as report of (71).	CEC	Vinylized the inner wall of the capillary/treatment the bare capillary with 1.0 M HCl for 30min/flushing with 1.0 M NaOH for 1 h/flushing and rinsing with water for 10 min and acetone for 20 min/drying under N_2 gas for 1 h/injection of TMSPM in acetone into the capillary/sealing the capillary with rubber septa at both ends/keeping in an oven at 25 °C for 24 h/washing the capillary with acetone/drying with N_2 gas for 30 min. **preparation of CD-GNP-modified monolithic column:** Sonicate polymerization mixtures GMA, EDMA, cyclohexanol, 1-dodecanol and AIBN/purging with N_2 for 10 min/filling the vinylized capillary with the polymerization mixture/sealing the capillary with rubber septa at both ends/start the polymerization reaction in a water bath at 40 °C for 40 h/rinsing the poly(GMA-co-EDMA) monolithic column with methanol/introduce thiol groups to the monolithic column/rinsing the thiol-modified column with H_2O for 20 min/flushing with aqueous solution of CD-GNP/standing the column for 1 h with sealing both ends/rinsing the column with H_2O/storing the column in a refrigerator at 4 °C.	[52]
Ovalbumin, myoglobin, lysozyme, and cytochrome C	Commercial hydroxyapatite NPs @ vinylized capillary	Commercial hydroxyapatite NPs	Commercial hydroxyapatite NPs.	CEC	Filling the vinylized capillary with a mixture of HEMA, EDMA, 1:3 cyclohexanol-dodecanol, and 1% AIBN, comprising of different amounts of hydroxyapatite nanoparticles/heating at 60 °C for 20 h/washing the monolithic columns with ACN and water and equilibrated in the mobile phase.	[53]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
Trp, Tyr, Phe, Gly-Trp, Gly-Tyr, Gly-Phe, ConA, α-Lac, β-Lg, and BSA	Fe_3O_4-COOH @ bare fused-silica	Fe_3O_4-COOH MNPs	Dissolvation of $FeCl_3.6H_2O$ in DEG/addition of NaOAc and Na acrylate/ stirrer at a speed of 500 rpm and 70°C until obtaining a homogeneous dark yellow solution/transfer into an autoclave and heating at 200 °C for 10 h/washing the products with ethanol and water/drying under N_2 atmosphere at 50°C for 12 h.	CEC	Rinsing bare fused-silica capillary with 1 M HCl (1 h), followed by deionized water (1 h), 1 M NaOH (1 h), and then deionized water (2 h) at a flow rate of 10 μL min^{-1}/washing with 20 mM Tris–HCl buffer (pH 8.3, 1 h)/rinsing the pretreated capillary with the PDDA solution for 1 h at a flow rate of 10 μL min^{-1}/preserving at room temperature for 20 h/rinsing the PDDA coated column by deionized water for 10 min/sonication a certain concentration of Fe_3O_4-COOH MNPs in H_2O for 30 min/ charging the Fe_3O_4-COOH MNPs in the PDDA coated OT column at a flow rate of 10 μL min^{-1}/preserving the column at 40 °C for 20 h/rinsing the column with deionized water/purging and drying under N_2 atmosphere at 50 °C for 1 h.	[64]
Tryptophan, threonine, and propranolol	GO-Fe_3O_4-BSA @ PDMS	GO-Fe_3O_4-BSA	Synthesize GO as reports of (185, 186). **Preparation of GO-Fe_3O_4 nanocomposites:** Sonication of GO in distilled water for 30 min/heating to 50 °C/purging with N_2/addition of $FeCl_3.6H_2O$ and $FeCl_2.4H_2O$ to the suspension and ultrasonicate for 20 min/addition of $NH_3.H_2O$ solution dropwise to the mixture with vigorous mechanical agitation/continue the reaction for 40 min at 50 °C protected by N_2/cooling the composites at room temperature/separation of composites magnetically by a commercial magnet/washing with double-distilled water for several times/dispersion the product in water to produce homogeneous GO-Fe_3O_4. **Conjugation of BSA onto magnetic GO-Fe_3O_4 nanocomposites:** Incubation of GO-Fe_3O_4 and BSA in TBE buffer at room temperature for about 24 h/precipitate the material and separation by a magnetic field for several times/resuspension the product in TBE buffer.	OT-CEC	Introduce the suspension of GO-Fe_3O_4-BSA into the PDMS microchip/after conjugation of GO-Fe_3O_4-BSA on the channel surface, flushing out the excessive solution in the channel/rinsing the microchannel with water for 5 min/before to use, filling the modified microchip with buffer solution and kept at 4 °C.	[67]
Alanine, leucine, lsoleucine, valine, methionine, and glutamic acid	MNP-β-CD and MNP-β-CD-IL	MNPs	---	CEC	Conditioning the capillary with 1 M NaOH, 0.1 M NaOH and distilled H_2O/purging the capillary with N_2 gas for 2 h/flushing the capillary with 1 mg/mL suspension of β-CDs functionalized MNPs for 10 min under a pressure of 940 mbar and H_2O for 2 min/repeating the mentioned steps four times.	[68]
Enantiomers of propranolol, ofloxacin, amlodipine, chlortrimeton, tropicamide, and atenolol	CM-β-CD-MMPs	MMPs	**Synthesize MMPs:** Reaction of aqueous solutions of $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ under a N_2 gas with vigorous stirring/heating at 80 °C/addition of NH_4OH/carrying out the reaction for 30 min at 80 °C/cooling at room temperature/washing the product using H_2O/drying MMPs in a vacuum oven for overnight. **Synthesize CM-β-CD-MMPs:** Activation of CM-β-CD using N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride and N-hydroxysuccinimide for 30 min/dispersion of APTES-MMPs in H_2O/adjusting pH to 7.0/separation of product after 24 h/drying the product using vacuum drying.	CEC	Washing the capillary with 1.0 M NaOH, 0.1 M NaOH, and H_2O for 30 min/purging with N_2 gas for 2 h/flushing the capillary with aqueous suspension of CD-MMPs for 5 min under a pressure of 940 mbar/washing the capillary via H_2O for 2 min.	[69]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
1,2-dihydroxybenzene, 1,3-dihydroxybenzene, 1,4-dihydroxybenzene, egg white, thiourea, naphthalene, biphenyl.	GNPs-MNPs	MNPs	**Synthesis of GNPs:** Mixing solutions of $HAuCl_4$, oleylamine, and cyclohexane (precursor solution)/stirrer the solutions at temperature of 10 °C under N_2 gas/dissolvation of TBAB complex in OAm and cyclohexane solutions/addition of TBAB solution to precursor solution/ addition of ethanol/centrifugation for 5 min/re-dispersion of gold particles in n-hexane for using. **Synthesis of Janus GNPs-MNPs:** Dissolvation of ferric chloride and sodium oleate in a mixture containing ethanol, H_2O and hexane/mixing $Fe(OL)_3$, Au seeds, OA and OAm into 1-octadecene/heating the solution to 120 °C for 20 min/reflux for 30 min at 320 °C/cooling at room temperature/separation of NPs by adding ethanol/centrifugation/re-dispersion of particles in n-hexane for using. **Synthesis of MNPs:** Fabrication of MNPs was done as mentioned above with the exception of Au addition.	OT-CEC	Filling the capillary with GNP-MNPs solution in n-hexane/washing the column with sodium phosphate buffer (pH 7.0) and 45% CH_3OH.	[70]
Chlorphenir amine, zopiclone, carvedilol enantiomers and dinitrophenyl-labeled amino acids of DL-Val, Leu, Glu, and Asp	β -CD-GNPs	GNPs	Mixing $NaBH_4$ in DMF with DMF solution of thiolated β-CD and $HAuCl_4$/ centrifugation the precipitate/remove free thiolated β-CD by washing the solid with DMF/washing further with ethanol and water/centrifugation and drying the solid at 60 °C under vacuum to give thiolated β-CD-GNPs.	CEC	---	[71]
Glycated proteins, BSA, and HTF	Silica GNPs	GNPs	GNP was prepared by Au (III) reduction using citrate according to Turkevich et al. report (187).	OT-CEC	Rinsing the capillary with 1 mL NaOH 1 M and 1 mL deionized water/drying at 180 °C for an overnight/rinsing the capillary with 100 µL of MPTMS, EtOH (96%), HCl (0.01 M) solution (7:2:1, v/v/v)/filling the capillary with this solution/keeping for 2 h at room temperature/forcing out of the excess of solution from capillary under argon pressure (500 kPa) for 10 min/drying at 120°C overnight/rinsing the capillary with 0.5 mL acetone and 0.5 mL MeOH at flow rate of 20 µL min^{-1}/drying at room temperature by argon flow (500 kPa) for 15 min/preparation GNPs-based stationary phase with 2 mL of freshly GNPs solution at flow rate of 16.6 µL min^{-1}/keeping the capillary for 2 days/pumping 1 mL of ultrapure water at flow rate of 20 µL min^{-1} through the capillary.	[72]
Propranolol enantiomers	---	GNPs	**Synthesis of GNPs by citrate-reduction method:** Mixing 0.75, 0.50, 0.30, and 0.20 mL aqueous solution of trisodium citrate with 50.0 mL boiled $HAuCl_4$ (0.01% w/w) solution/stirring at boiling points of solutions for 30 min/cooling at room temperature/keeping the GNP solutions at 4 °C. **Synthesis of GNPs by hydroborate-reduction method:** Mixing 0.40 mL of 1% trisodium citrate and 0.15 mL of 0.075% $NaBH_4$ solution containing 1.0% trisodium citrate with 100.0 mL of 0.01% $HAuCl_4$ at room temperature/stirring	TLC	Insertion the TLC plates in a chamber containing GNPs in solution of acetone–water/drying the plate at 80 °C for 4-5 min/dipping the dried plates on the solution of L-cys (pH ≥ 6)/developing the plate in a TLC chamber containing 10.0 mM Cu^{+2} acetate in 70% ethanol and water/dipping the plate on GNPs in solution of acetone–water/drying the plate at 80 °C.	[73]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
			the mixture for 30.0 min at room temperature/keeping the solution at 4 °C.			
Benzene, toluene, ethylbenzene, cumene, *n*butylbenzene, *n*-amylbenzene, hydroquinone, resorcinol, catechol, thymidine , uridine, cytosine, inosine, xanthine, 6-chlorouracil, DL-leucine, L-tyrosine, proline, glycine, 3-nitro-L-tyrosine, and L-glutamic acid	GNPs @ GO-silica	GNPs	**Synthesis of GNPs:** Boiling $HAuCl_4$ aqueous solution under constant stirring/addition of trisodium citrate aqueous solution/continue stirring for another 2 min/ heating for 15 min. **Synthesis of GNPs@GO-SiO_2:** Addition of dried aminopropylsilica particles to GO solution under ultrasonic treatment for 5 min/stirrer at 80 °C for 12 h/washing the GO-SiO_2 with deionized water and then dried/suspension of GO-SiO_2 to toluene/addition of an excess of thiopropyltriethoxysilane/stirrer the suspension and refluxed for 48 h/washing the sulfhydrylpropyl @ GO-SiO_2 with toluene, ethanol-water and deionized water in turn/drying under vacuum for 12 h at 60 °C/addition of sulfhydrylpropyl @ GO-SiO_2 to the GNPs solution under ultrasonic for 20 min/stirrer for 3 h/drying GNPs @ GO-SiO_2 under vacuum at 60 °C.	HPLC	Packing the columns using slurries of the GO@SiO_2 and GNPs@GO-SiO_2 in CCl_4/utilization of *n*-hexane as the propulsive solvent/after preparation of GNPs@SiO_2 column, *n*-hexane-isopropanol was used for slurrying and propelling the GNPs-SiO_2.	[74]
Amino acids	BSA-GNPs @ silica	GNPs	The GNPs were synthesized based on the sodium citrate synthesis method as report of (188).	CEC	Preparation of the monolithic silica capillary column as report of [189].	[75]
Nucleic acid bases, nucleosides, ABs, PAAs, N,N-dimethylaniline, 4-chloroaniline, aniline, 4-ethylaniline, n-ethylaniline, 4-bromoaniline, 4-isopropylaniline, and 3-chloroaniline	MWCNT-COOH @ fused silica capillary and MWCNT-SO_3H @ fused silica capillary coated with SDS	MWCNT-COOH and MWCNT-SO_3H	**MWCNT-COOH:** Addition of MWCNTs to a mixture of concentrated H_2SO_4 and HNO_3/sonication in a water bath for 5 h at room temperature/dilution the reaction mixture with deionized water/centrifugation/washing with an excess of water/after drying in an oven MWCNT-COOH was achieved. **MWCNT-SO_3H:** Addition of MWCNTs to H_2SO_4/ mixed for 18 h at 250°C under N_2/cooling the reaction mixture down to room temperature/washing the product with water/centrifuge and draying at 120 °C in an oven for 12 h to obtain sulfonated MWCNTs-SO_3H.	CEC	Addition of unmodified and functionalized MWCNTs to electrolyte solution/sonication/flushing the mixture with H_2O for 10 min, 0.1 M NaOH for 30 min to the new capillary/running the electrolyte for 15 min/before analysis flush the capillary with 0.1 M NaOH for 1 min, H_2O for 1 min and running electrolyte for 2 min.	[81]
Herbicides, barbiturates, dansyl-DL-amino acids, dipeptides, and proteins	MWCNT-COOH @ fused silica capillary, MWCNT-SO_3H @ fused silica capillary and MWCNT-OH @ fused silica capillary coated with SDS	MWCNT-COOH, MWCNT-SO_3H, and MWCNT-OH	Refer to Ref (81).	CEC	Refer to Ref [81].	[84]
Alkylbenzenes, polycyclic aromatic hydrocarbons, amines,	ODS-GO @ SiO_2	GO	Addition of GO to deionized water/ultrasonic for 1 h/addition of aminopropylsilica particles to the GO dispersion solution under ultrasonic for 5 min/stirrer at 80 °C for 12 h for the bonding of GO/washing the GO-SiO_2	HPLC	Applying 40 MPa pressure for packing the column via slurries of SP using MeOH as the propulsive solvent.	[95]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
and phenolic compounds			via water and drying/addition of GO-SiO_2 into dry toluene under ultrasonic for 5 min/addition of octadecyltrichlorosilane/stirrer the suspension and refluxed for 48 h/washing with toluene and ethanol/drying ODS-GO-SiO_2 under vacuum at 60 °C for 12 h.			
Ovalbumin, ovotransferrin, ovomucoid, ovoflavoprotein, lysozyme, and avidin	G and GO @ fused-silica	G and GO	Preoxidized graphite powder in a concentrated mixture of H_2SO_4, $K_2S_2O_8$ and P_2O_5 at 80°C for 4.5 h/dilution with pure water/washing with water/drying at room temperature/addition of preoxidized graphite into cold concentrated H_2SO_4/addition of $KMnO_4$ under stirring with an ice-bath to control the temperature below 20 °C/stirrer for 4 h/addition of H_2O_2 and pure water to terminate the reaction/filtering/washing with 1.2 M HCl solution/drying finally to give a brown solid/dispersion in water /ultrasonication for 10 min to obtain a clear dispersion of GO.	CEC	**For column of GO:** Flushing a bare fused silica capillary with 1 M NaOH (3 h), water (30 min), 1 M HCl (30 min), water (1 h), and acetone (30) min/drying with N_2/flush the capillary with 200 µL of 3-AMDS toluene solution (1 %)/storing the capillary at 25°C/flushing with $N_2$for 10 min/flow the capillary with 100 µL of 0.2 wt % aqueous GO/sealing at both ends and heating at 70°C for 2 h/purging with N_2/washing with water (30 min). **For column of G:** Condition the GO-coated column in the GC injector under N_2 at 60 °C for 2 h/inject mixture of 5.0 mL of H_2O, 20.0 µL of hydrazine (35 wt % in H_2O) and 35.0 µL of ammonia solution (28 wt % in H_2O) into the GO-coated column/sealing at both ends and heating at 70 °C for 18 h to obtain the column of G.	[96]
Lysozyme, cytochrome C, and α-chymotrypsinogen A	P-*f*SiO_2 @ C_{18}	Fibrous mesoporous silica	Dissolvation of CTAB and urea in water/addition of cyclohexane and isopropanol to the solution/vigorous stirring/addition of TEOS dropwise to the mixed solution/vigorous stirring for 30 min at room temperature/heating the reaction mixture up to 72°C, and maintained for 10 h/cooling the mixture at room temperature/centrifugation and isolating the silica/washing with distilled water and acetone/drying under air for 24 h/transferring the materials to 0.6% (w/v) NH_4NO_3 ethanol solution/keeping at 60°C for 4 h/growing *f*SiO_2 onto 300 nm initiator modified *f*SiO_2 via atom transfer radical polymerization (ATRP). **Synthesis of initiator-modified *f*SiO_2:** Suspend *f*SiO_2 in 50 mL of APTES dry ACN solution for 24 h at room temperature/collection the amine-modified *f*SiO_2/rinsing with ACN via repeated centrifugation/suspension of the amine-functionalized particles in dry dichloromethane solution of dry triethylamine, 2-bromoisobutyryl bromide, and amount of DMAP for 12 h at room temperature/isolation the initiator-modified particles/rinsing with dichloromethane/addition of PDMAEMA onto the *f*SiO_2 in a mixture of degassed MeOH and water, containing DMAEMA, 2,2´-dipyridyl, $CuCl_2$, and CuCl at room temperature/quenching and rinsing with MeOH and water/drying at 60°C for 2 h to achieve P-*f*SiO_2/modification of P-*f*SiO_2 nanoparticles with 10% octadecyltrichlorosilane in toluene at 110°C for 24 h to obtain P-*f*SiO_2@C_{18}.	CEC	Rinsing and filling fused silica capillary with 1 M NaOH for 30 min, 1 M HCl for 30 min, acetone for 30 min/addition of PDMAEMA-modified mesoporous silica nanoparticles (P-*f*SiO_2) to 1 mL ethanol/transportation of suspension into the pretreated capillary/after evaporation of solvent, rinsing the column with methanol and water/drying under N_2 (denoted as P-*f*SiO_2column)/flushing with 10% octadecyltrichlorosilane in toluene/sealing by two GC septa at both ends/heating at 110°C for 24 h/rinsing with methanol and water, respectively/drying with N_2 (denoted as P-*f*SiO_2@C_{18} column)/total length of the open-tubular column was 65 cm with an effective length of 45 cm.	[99]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
Angiotensin I, oxytocin, bradykinin, LHRH, and met-enk	GNPs @ fused-silica	GNPs	Dissolvation of $HAuCl_4.3H_2O$ in water/boiling this solution in a round bottomed flask under stirring/addition of sodium citrate solution /after for 20 min boiling, remove the heat and continue stirring for 15 min/centrifuge the suspension of nanoparticle/remove the supernatant and the remaining NPs were dispersed in water again.	OT-CEC	Rinsing the untreated capillary 1M NaOH and water/drying with N_2 gas/coating the capillary with rinsing for 30 min via HCl-MeOH (1:1)/flushing with methanol/drying with N_2 gas/passing the APTES through the fused-silica capillary under pressure for 15min/rinsing the capillary with ethanol and drying in N_2 stream/wringing the GNPs suspension through the APTES-modified capillary for 2 h and washing with water.	[104]
Chlorphenir amine, zopiclone, and tropicamide	β -CD-GNPs	GNPs	CD-GNPs were synthesized using a three-step process, as report of (71).	OT-CEC	Rinsing the untreated capillaries using 1M NaOH (5min), 0.1M NaOH (1 h) and deionized water (30 min)/ filling and flush the capillaries using PDDA solution (2%, w/w) comprising 0.1M NaCl (1 h), CD-GNPs solution (1.8 mg/mL, 1 h)/washing the capillaries with deionized water for 5 min/keeping the coated capillary columns in solution of CD-GNPs at 4 °C after utilize.	[119]
Amino acids	Cellulose tris(3-chloro-4-methylphenylcarbanate) as reports of (190, 191)/dissolvation the polysaccharide derivative in tetrahydrofuran/coating onto aminopropylsilanized spherical silica particles.	---	Synthesize cellulose tris(3-chloro-4-methylphenylcarbamate) @ fused-silica	CEC and nano-LC	Preparation the packing was done as report of [192].	[120]
Ovalbumin, ovotransferrin, ovomucoid, lysozyme, avidin, and neutral PAHs	GNPs-PEM	GNPs	Synthesis was done based on the reports of Gittins et al. and Freeman et al. (193, 194).	CEC	**Type I capillary:** Condition the capillary with 1.0 M NaOH for 30 min/washing with deionized water for 15 min/rinsing the capillary with deposition solutions (5% (w/v) polyelectrolyte and 0.1 M NaCI)/modification of capillary with a cationic layer of PDADMAC and an anionic layer of PSS/rinsing the capillary with GNP solution for 30 min. **Type II capillary:** Direct adsorption of GNPs onto the capillary wall. **Type Ill capillary:** Adsorption of negatively charged GNPs to the capillary modified with 3 layers of polyelectrolyte (PDADMAC/PSS/PDADMAC). derivatization of 3 types of capillary columns with n-octadecanethiol/remove the additional n-octadecanethiol by pumping hexane and then flushing with EtOH and distilled water.	[121]
Long-stranded DNA	GNPs-PEO	GNPs	**Synthesis of GNP:** Heating solution of 0.01% $AuCl_4$/addition of 1% trisodium citrate/heating and reflux for 8 min/change the color indicating the formation of GNP/cooling at room temperature. **Synthesis of GNPP:** Addition of 0.5% PEO solution to GNP/keeping the solution for 1 h/centrifugation at for 10 min/washing the pellet with 10 mM GC buffer (pH 7.0)/centrifugation at for 10 min/disperse GNPP in 10 mM GC buffer.	CE	Flushing the capillary with deionized H_2O/filling the capillary with the GNPPs by applying a low pressure.	[130]
Long-stranded DNA	GNPs-PEO	GNPs	**Synthesis of GNP:** The GNPs were synthesized based on the report of (130). **Synthesis of GNPP:** mixing the GNPs with PEO (2 and 8 MDa)/addition of PEO solutions (0.1–20 μL) to various concentrations of the GNPs solutions/equilibration at room temperature and pressure overnight/mixing with a suitable concentration of ethidium bromide.	CE	Flushing the capillary with deionized H_2O/filling the capillary with the GNPPs by applying a low pressure.	[133]
α-chymotrypsinogen, ribonuclease A,	DDAB-capped GNP @ fused-silica	GNPs	**Preparation of GNPs:** Mixing the solution of $HAuCl_4$ with DDAB solution under vigorous stirring/addition of $NaBH_4$ solution at and mixing for 2 min/changing the color from pale yellow	CE	Flushing the untreated capillary with 0.5 M NaOH 10 min, then by water for 1 min/rinsing the capillary by 0.1 mM DDAB or 10 min with GNPs/filling the capillaries with the 0.1 mM DDAB, GNPs, and GNPPs by applying a low pressure.	[134]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
trypsinogen, cytochrome c, lysozyme, BSA, carbonic anhydrase, ovalbumin, myoglobin, and α-lactalbumin			to wine, showing the formation of the GNPs. **Preparation of GNPPs:** Mixing the GNPs with PEO/addition of 0.5% PEO solutions to different concentrations of the GNP solutions/equilibration the solution at ambient temperature and pressure for an overnight.			
Ephedrine and norephedrine enantiomers	BSA-GNPs	GNPs	**Synthesize GNPs:** Mixing sodium citrate solution with $HAuCl_4$ solution at 80 °C under vigorous stirring/cooling at room temperature with stirring. **Conjugation of BSA-GNP:** BSA-GNP conjugation was performed as report of [188].	CE	Cleaning the glass microdevice using H_2SO_4-H_2O_2 bath at 60 °C/washing with deionized water/rinsing with 1M NaOH, deionized water and methanol/purging the channel using N_2 gas/injection mixture of MPTMS-MeOH into the dried microchannels and stood for 24 h/washing the MPTMS-coated microchannels via methanol and deionized water/injection the conjugation of BSA-GNP/flushing out the microchannels with deionized water/storing the microdevice in deionized water at 4 °C before utilization.	(156)
Chlorphenamine, brompheniramine, pheniramine, and zopiclone enantiomers	β -CD-GNPs	GNPs	Synthesis of mercapto-β-cyclodextrin gold nanoparticles was performed as the report of [195].	OT-CEC	Treatment of the capillary with NaOH solution for 2 h/ washing the column with HCl, H_2O, and acetone for 30 min/purging the capillary with N_2 gas for 30 min at room temperature/derivatization of the inner wall of capillary with (3-mercaptopropyl) trimethoxysilane/rinsing the functionalized capillary with propan-2-ol/annealing the capillary at 110 °C for 10 min in the GC oven under N_2 gas/pushing the sol-gel solution into the functionalized capillary at a length of about 40 cm via a syringe/bring out the excess solution of sol-gel via N_2 gas for 5 min/drying the coated capillary at a temperature of 120 °C under N_2 gas for overnight/washing the capillary with acetone, methanol and water/injection of the SH-β-CD-GNPs to the thiol containing inner surface/removing the excess solution from the capillary using distilled H_2O/keeping the capillary in distilled H_2O until use.	[163]
Two pairs of α-tetralones derivatives enantiomers, enantiomers of tramadol hydrochloride and zopiclone	β -CD-GNPs	GNPs	Synthesis of OTs-β-CD (II) as report of (196)/synthesis of 6-deoxy-p-methoxy-α-benzylthio-β-cyclodextrin (III)/synthesis of 6-deoxy-6-p-methoxy-α-toluene thiol-carboxymethyl-β-cyclodextrin (IV)/synthesis of 6-deoxy-6-mercapto-carboxymethyl- β -cyclodextrin (V). **Synthesis of GNPs:** synthesis of GNPs was done as report of (195).	OT-CEC	---	[164]
Propranolol, esmolol, bisoprolol and sotalol	Carboxymethyl-β-CD GNPs	GNPs	Stirring aqueous solution of $HAuCl_4 \cdot 3H_2O$ for 1 min/addition of sodium citrate/addition of $NaBH_4$/stirrer for 5 min.	OT-CEC	Flushing the column using NaOH (1 h), H_2O (0.5 h), HCl (0.5 h), H_2O (0.5 h), MeOH (0.5 h)/drying at 100 °C for 2 h/rinsing the capillary with MPTMS/sealing both ends of column/insertion the capillary in the water bath at 55 °C for 12 h/rinsing the silanized capillary with MeOH and drying under N_2 gas/pumping the GNPs solution into the capillary at room temperature for 5 h/flushing with H_2O/flushing with L-cysteine for 5 h/rinsing the capillary with H_2O/pumping the EDC through the L-cysteine-GNPs capillary for 1 h/flushing the capillary with carboxymethyl-β-cyclodextrin for 5 h/rinsing the capillary with H_2O.	[165]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
Cysteine, cysteinylglycine, homocysteine, glutamylcysteine, and glutathione	Brij 35-capped GNPs	GNPs	Stirring sodium citrate in a flask fitted with a reflux condenser /addition of 10% $HAuCl_4$/maintaining at the boiling point with continuous stirring for 15 min/cooling to room temperature/addition of 10% Brij 35 in methanol.	HPLC	---	[170]
Nucleosides, sulfa compound, and safflower	CNPs @ silica	CNPs	Reflux waste soot in nitric acid for 15 h with stirring/cooling the solution and neutralized by NaOH/centrifugation for 15 min/dialyze the reddish-brown supernatant against distilled water for 72 h to obtain purified CNPs.	HPLC	Suspension CNPs in anhydrous DMF/adjusting pH to 4.5 by formic acid/stirrer the mixture/addition of NHS and EDC to solution to activate the surface carboxyl groups of CNPs/addition of APTMS dropwise after 30 min/stirrer at room temperature for 12 h/addition of silica gel dispersion into the solution/addition of CPTMS and ammonia solution to the mixture/heating under a N_2 atmosphere at 100 °C for 15 h/filtering the solution/washing with DMF, water, methanol and acetone/drying under vacuum at 40 °C overnight/packing the column with new separation material/using MeOH as the packing solvent at pressure of 60 MPa.	[171]
Chloroethyl naproxenate	ZrO_2-SiO_2 support, C_{18}-ZrO_2-SiO_2 and CDMPC@ ZrO_2-SiO_2	ZrO_2-SiO_2	Washing silica with acid, alcohol, and deionized water/vacuum drying/filming silica core particles with surfactant/addition of silica particles into a solution of 0.05 mol L^{-1} SDS for 30 min/washing procedure to eliminate the physically adsorbed SDS/drying SDS-SiO_2/depositing zirconia sol on SDS-SiO_2/stirrer for 3 h/washing monolayer of ZrO_2-SDS-SiO_2 and then dried/repeating procedures several times to make multilayers of ZrO_2-SDS-SiO_2/drying at 120 °C and sinter at 560 °C/transferring multilayers of ZrO_2-SDS-SiO_2 to NPs of ZrO_2-core SiO_2 particles/the LbL self-assembly method allows the fabrication of the preferred layers of ZrO_2 NPs on SiO_2 spheres.	HPLC	Synthesize C_{18}- ZrO_2-SiO_2 by reaction between ZrO_2-SiO_2 and octadecyltrichlorosilane/continuously stirrer with toluene reflux/washing procedure with toluene and methanol/drying the product in a vacuum oven/filling the stainless steel column of 150 × 4.6 mm i.d. at 45 Mpa pressure. **Chiral polysaccharide polymer SP CDMPC @ ZrO_2-SiO_2:** Synthesize CDMPC by derivation of microcrystalline cellulose using 3,5-dimethylphenyl isocyanate based on report of Okamoto et al. [197]/fabrication of chiral stationary phase of CDMPC @ ZrO_2-SiO_2 according to the report of Castells et al. (198)/filling the column of 150 × 4.6 mm i.d. at 50 Mpa pressure.	[180]
Ibuprofen, flavanone, 6-hydroxyflavanone, malathion, cypermethrin, olaquindox, chlorpyrifos, fenthion, and diazinon	GNP @ silica	GNPs	Addition of $HAuCl_4.4H_2O$ and trisodium citrate to a flask under stirrer/addition of borohydride solution/centrifugation/re-dispersion with H_2O.	HPLC	**Preparation particles of SiO_2-Au:** **Step 1:** Mixing aqueous solutions of GNPs and 3-aminopropyltrimethoxysilane-bonded silica at room temperature for 10 h/centrifugation/washing with H_2O/re-dispersion in H_2O. **Step 2:** Addition of K_2CO_3, H_2O, and $HAuCl_4$ solutions to a beaker/stirrer/aging in the dark for 24 h. **Step 3:** Mixing solutions of steps 1 and 2/stirrer with hydroxylamine hydrochloride/dispersion the precipitate in H_2O for using. **Synthesis of 3,5-dimethylphenyl isocyanate derivative of cellulose:** Drying cellulose in a three-necked round-bottomed flask at 80 °C for 4 h under vacuum condition/addition of anhydrous pyridine and an excess of 3,5-dimethylphenyl isocyanate/reflux the mixture with constant stirring for 24 h/filtration, washing, and drying the solid. **Coating of 3,5-dimethylphenyl isocyanate derivative of cellulose on the surfaces of SiO_2-Au:** Dissolvation of 3,5-dimethylphenyl isocyanate derivative of cellulose in tetrahydrofuran/keeping for overnight/addition of SiO_2-Au. **Preparation of SiO_2-cellulose derivative CSP:** Dissolvation of 3,5-dimethylphenyl isocyanate derivative of cellulose in tetrahydrofuran/keeping for overnight/addition of SiO_2.	[181]
Progesterone and testosterone	GNPs-APTS @ silica gel plate	GNPs	Addition of $HAuCl_4$ to deionized water/boiling with stirring under reflux/addition of trisodium citrate	TLC	**Preparation of GNPs-APTS @ silica gel:** Addition of silica gel to concentrated HCl/heating under reflux for 2 h/washing with deionized	[182]

Continued Table 1. Description of nano-stationary phases of analytical techniques.

Analyte	Stationary phase of column/plate	Nanoparticle	Conditions of making nanoparticles	Technique	Preparation of coated columns	Ref.
			under continuous stirring/keeping the reaction in the same condition for 5 minutes after changing the color/cooling to room temperature via an ice bath.		water/centrifugation/placing activated silica gel in an oven at 100 °C for 24 h /addition of silica powder to a solution of 1% (v/v) APTS in ethanol/stirrer for 2 h and keeping for 24 h/decantation and annealing at 100 °C in an oven for 24 h to prepare APTS-silica gel/mixing a solution of GNPs with APTS-silica gel and stirrer for 2 h/washing and decantation several times with deionized water to remove excess amounts of GNPs/placing GNPs-APTS modified silica gel in an oven at 50 °C for 24 h. **Preparation of GNPs @ silica gel:** Mixing activated or plain silica gel with the colloidal GNPs under vigorous stirring for 2 h/washing and decantation procedure as mentioned for GNPs-APTS-silica gel/placing the prepared GNPs @ silica gel at 50 °C in an oven.	

CLC: Capillary liquid chromatography/GNP: Gold nanoparticle/BSA-GNP: Bovine serum albumin-gold nanoparticle/TLC: Thin layer chromatography/β-CD: β-cyclodextrin/SWNT: Single-wall nanotube/BSA: Bovine serum albumin/HTF: Human transferring/polyDVB: Poly(divinylbenzene)/polyEDMA: Poly(ethylene glycol dimethacrylate)/EDMA: Ethylene glycol dimethacrylate/DVB: Divinylbenzene/HD: Hexadecane/AIBN: Azobisisobutyronitrile/SDS: Sodium dodecylsulfate/APS: Ammonium persulfate/TEMED: N,N,N′,N′-tetramethylethylenediamine/PEGDA: Poly(ethyleneglycol) diacrylate/MNPs: Magnetic nanoparticles/DEG: Diethylene glycol/γ-MAPS: 3-(trimethoxysilyl)propylmethacrylate/AIBN: 2,2′-azobisisobutyronitrile/NPs: Nanoparticles/γ-MAPS: γ-methacryloxypropyltrimethoxylsilane/GMA: Glycidyl methacrylate/GA: Glutaraldehyde/APTES: 3-Aminopropyltriethoxysilane/CTAB: Hexadecyl trimethyl ammonium bromide/TEOS: Tetraethyl orthosilicate /DMAP: 4-Dimethylaminopyridine/ABs: Alkylbenzenes/PAAs: phenyl alkyl alcohols/HEMA: 2-Hydroxyethyl methacrylate/CDMPC: Cellulose tris(3,5-dimethylphenylcarbamate) polymer/MNP-β-CD-IL: Magnetic nanoparticles-β-cyclodextrin-ionic liquid/MMPs: Magnetic microparticles/CSP: Chiral stationary phase/ODS-GO-SiO_2: Octadecylsilane-graphene oxide-SiO_2/CNP: Carbon nanoparticle/CPTMS: 3-(chloropropyl)trimethoxysilane/NHS: N-hydroxysuccinimide/EDC: 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride/GMM: Glyceryl monomethacrylate/FSNPs: Fumed silica nanoparticles/TMSPM: 3-(Trimethoxysilyl)propyl methacrylate/MFSNP: Methacryloyl fumed silica nanoparticle/DODCS: Dimethy-loctadecylchlorosilane/PEO: poly(ethylene oxide)/GNPPs: GNPs-polymer composites/TBE buffer: mixtures of Tris, boric acid, and EDTA/PDMS: poly(dimethylsiloxane)/MPTMS: (3-mercaptopropyl)-trimethoxysilane/DDAB: didodecyldimethylammonium bromide/VBC: vinylbenzyl chloride/APTS: 3-triethoxysilyl propylamine/GC: glycine-citrate/PEO: Poly(ethylene oxide)/PEM: Polyelectrolyte multilayer/PSS: Poly(sodium-4-styrenesulfonate)/PDADMAC: Poly(diallyldimethylammonium chloride)/DMAP: 4-dimethylaminopyridine.

new SP gave a better resolution in comparison with C_{18} commercial column. In the following, Liang et al. in another investigation applied GNPs onto the surface of GO@silica as a new SP of HPLC [74]. Compared with the GO@silica phase, GNPs@silica phase displayed good resolutions for alkylbenzenes, isomerides, amino acids (DL-leucine, L-tyrosine, proline, glycine, 3-nitro-L-tyrosine, and L-glutamic acid), nucleosides (thymidine, uridine, cytosine, inosine, and xanthine), 6-chlorouracil, and nucleobases due to the combination of hydrophilicity, large π-electron structures, hydrophobicity, and coordination groups, which resulted in a unique separation.

Incorporation of FSNPs into a monolithic column of polymethacrylate including glyceryl monomethacrylate (GMM) and EDMA was done to prepare a novel monolithic column for nucleosides of adenosine, uridine, cytidine, inosine and guanosine, hydroxybenzoic acids and nucleotides using HILIC [36]. Decreasing the pH of aqueous mobile phase was resulted in reducing the electrostatic repulsion and enhancing the retention time of acidic components. The best separation of nucleotides in less than 10 min was achieved via gradient elution with minimization of pH and ACN volume of mobile phase. Flame hydrolysis of silicon tetrachloride in a gas flame of oxyhydrogen at 1000 ˚C led to fabricate fumed silica with amorphous, extremely dispersed, nonporous, and hydrophilic properties [172, 173]. In the following, Aydoğan and Rassi in alternative investigation modified FSNPs with 3-(trimethoxysilyl)propylmethacrylate (TMSPM) to make the hybrid MFSNP monomer. The obtained MFSNP was combined with GMM and EDMA in a solvent comprising of cyclohexanol and dodecanol to initiate copolymerization of MFSNP, GMM and EDMA in a stainless steel column. Octadecyl ligand was used to incorporate the silanol groups of the hybrid monolith, which finally obtained the hybrid poly(GMA-EDMA-MFSNP)-octadecyl with hydrophobic interactions. Alkylbenzenes, phenolic compounds, aniline, 4-methylaniline, N-methylaniline, N-ethylaniline, 3,4-dichloroaniline, lysozyme, ribonuclease A, carbonic anhydrase isozyme II, cytochrome C, α-chymotrypsinogen A, and myoglobin were chromatographed on the monolithic column.

Pharmaceutical

To analyze the chiral compounds, the application of analytical columns may be inappropriate because of high costs of chiral SP, great consumption of solvents, and a destructive environmental effect. Thus, various miniaturized methods such as capillary liquid chromatography (CLC) and nano-LC have been designed for the separation of chiral compounds [144, 174-176]. Recently, chiral selectors with different separation mechanisms have been demonstrated. For example, glycopeptide antibiotics as a chiral marker for amino acids, peptides, herbicides, and drugs displayed very high enantio resolution [177-179].

The LbL self-assembly procedure was applied to make NP of ZrO_2-SiO_2 as SP of HPLC [180]. Despite the silica support, zirconia is very stable over the pH of 1-14, whereas the availability and improvement of specific surface area and pore volume of zirconia is much lower than silica. Therefore, to enhance the surface area of zirconia-based support, Dun et al. used LbL self-assembly technique. Both of ZrO_2-SiO_2 support and SP of C_{18}- ZrO_2-SiO_2 revealed outstanding chemical stability and good permeability. Moreover, cellulose tris(3,5-dimethylphenylcarbamate) polymer (CDMPC) was coated on ZrO_2-SiO_2 support for discrimination of chiral compounds.

In 2020, Li and coworkers prepared GNPs with particle size of 10-15 nm as a polysaccharide-based CSP for separation of ibuprofen using normal phase liquid chromatography. Also, six chiral pesticides, flavanone, and 6-hydroxyflavanone were investigated. It can be concluded that the -OH groups of cellulose provided a strong electrostatic attraction and hydrogen bond interaction, which resulted in long retention time of ibuprofen [181].

The important information of described analyses is presented in Table 1.

THIN LAYER CHROMATOGRAPHY

In addition to the mentioned methods, application of TLC has increased newly, because of low-cost, rapid, efficient resolution, and low consumption of solvents. Therefore, due to the advantages of NPs and TLC technique, some amendments for SPs have been applied to resolve peak tailing of chromatograms and give a better resolution. To do this, surface modification of a commercial TLC plate for direct detection of propranolol enantiomers was performed with GNPs as the first- and L-cysteine (L-cys) as the second layer, based on the ligand-exchange mechanism [73]. Ligand-exchange chromatography as one of the direct chromatographic techniques is used for a superior chromatographic enantioseparation. High attraction of thiol group of L-cys toward GNPs resulted in an excellent adsorption on the surface of plate.

Another method has been developed to modify silica gel with GNP grafted 3-triethoxysilyl propylamine as a novel SP for separation of progesterone and testosterone [182]. Modification of silica gel SP using GNPs provides less baseline interferences, causing a suitable interaction between SP and steroid hormones, and gives a good sensitivity. LOD values of 0.16 and 0.13 ng/spot and LOQ values of 0.51 and 0.40 ng/spot for progesterone and testosterone were achieved, respectively [182]. The main conditions are accessible in Table 1.

CONCLUSION

In the present literature survey, several NPs as SPs of electrophoretic and HPLC techniques were reviewed such as GNPs, CNTs, MNPs, GO, and G for separation of biomolecules and chiral drugs. Also, owing to importance of the analysis of biomolecules and chiral drugs, some studies have been allocated to modify columns using chiral selectors. Moreover, some separations were performed using miniaturized techniques because of high-throughput screening, inexpensive, great separation efficiency, and satisfied resolutions. It was revealed that high ratio of analyses has been performed using GNPs and electrophoretic procedures. GNPs in analysis of biomolecules have attracted a considerable attention, due to the formation of strong covalent bonds between GNPs and thiol groups in structure of biomolecules. Finally, it is concluded that NP-based SPs will play a significant role in separation studies as well as the bioanalytical identifications.

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CONFLICTS OF INTEREST

The authors announce that there are no conflicts of interest.

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