REVIEW

Potential role of gold nanoparticles for improved analytical methods: an introduction to characterizations and applications

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Abstract Nanoparticle-based material is a revolutionary scientific and engineering venture that will invariably impact the existing analytical separation and preconcentration for a variety of analytes. Nanoparticles can be regarded as a hybrid between small molecule and bulk material. A material on the nanoscale produces considerable changes on various properties, making them size- and shape-dependent. Gold nanoparticles (Au NPs), one of the wide variety of core materials available, coupled with tunable surface properties in the form of inorganic or inorganic-organic hybrid have been reported as an excellent platform for a broad range of analytical methods. This review aims to introduce the basic principles, examples, and descriptions of methods for the characterization of Au NPs by using chromatography, electrophoresis, and self-assembly strategies for separation science. Some of the latest important applications of using Au NPs as stationary phases toward open-tubular capillary electrochromatography, gas chromatography, and liquid chromatography as well as roles of run buffer additive to enhance separation and preconcentration in the field of chromatographic, electrophoretic and in chip-based systems are reviewed. Additionally, we review Au NPs-assisted state-of-the-art techniques involving the use of micellar electrokinetic chromatography, an online diode array detector, solid-phase extraction, and mass spectrometry for the preconcentration of some chemical compounds and biomolecules.

Abbreviations

APTMS	3-aminopropyltrimethoxysilane
Au NPs	gold nanoparticles
Au NPPs	Au NPs/polymer composites
BSA	bovine serum albumin
CE	capillary electrophoresis
CEC	capillary electrochromatography
C18	*n*-octadecanethiol
DA	dopamine
DAD	diode array detector
DDAB	didodecyldimethylammonium bromide
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA
EDL	electric double layer
EOF	electroosmotic flow
EP	epinephrine
GC	gas chromatography
HPLC	high-performance liquid chromatography
I.D.	inside diameter
LBL	layer-by-layer
LPEI	linear polyethylenimine
MALDI	matrix-assisted laser desorption/ionization
MEKC	micellar electrokinetic chromatography
µHPLC	capillary HPLC
MPN	monolayer-protected Au NPs

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MPTMS	3-mercaptopropyltrimethoxysilane
MS	mass spectrometry
NBAMS	nanoprobe-based affinity mass spectrometry
NFCE	nanoparticle-filled capillary electrophoresis
NPs	nanoparticles
OD	outer diameter
ODA	octadecylamine
OH-PAHs	monohydroxy-PAHs
OTCEC	open-tubular capillary electrochromatography
PAG	polyacrylamide gel
PAGE	polyacrylamide gel electrophoresis
PAHs	polycyclic aromatic hydrocarbons
PDDAC	poly(diallyldimethylammonium chloride)
PDMS	polydimethylsiloxane
PEO	poly(ethylene oxide)
pI	isoelectric point
PMMA	poly(methyl methacrylate)
PVP	poly(vinyl pyrrolidone)
REPSM	reversed electrode polarity stacking mode
SEC	size exclusion chromatography
SEM	scanning electron microscope
SPE	solid-phase extraction
SPNE	solid-phase nanoextraction
ssDNA	single-standed DNA
TOF	time-of-flight

Introduction

Nanoparticles (NPs), i.e. particles with structures approximately 1–100 nm in size, have significant impact in many scientific fields, including chemistry, material sciences, biology, medicine, and electronics [1–10]. The physical, material, and chemical properties of NPs are directly related to their intrinsic compositions, apparent sizes, and extrinsic surface structures [11–16]; therefore, the design, synthesis, characterization, and applications of nanostructures are critical aspects for the emerging field of nanomaterials. The concept of nanoparticle-assisted sample separation and preconcentration plays important roles in many analytical procedures. Whilst primarily associated with increasing the concentration of analytes, the approach provides a number of benefits, ranging from the removal of interfering species to a potentially beneficial change in the matrix composition [17]. The potential capability of NPs has been extensively studied in separation science during recent years, and many advances have been achieved toward chromatographic and electrophoretic systems for a couple of separation components and preconcentration media. The NPs serve either as inner surface coating in permanent or dynamic mode, or as a pseudostationary phase added to the buffer and used in partial filling or continuous filling form. Several reviews on this topic have summarized the latest developments in the

characterizations and applications of NPs [18–24]. Among those NPs are polymers, silica, fullerenes, carbon nanotubes, titanium oxide, silver nanoparticles, and gold nanoparticles (Au NPs). This review is focused only on Au NPs with a highly promising and reliable utilization due to their high surface-to-volume ratio, long-term stability, easy synthesis, and favorable chemical modification.

Cold-hammering was used in the late Stone Age to produce plates of gold for ornamental purposes, and the bulk gold has always been synonymous with beauty, power, and wealth. In contrast, the scaling-down of bulk gold to nanometer size, i.e., Au NPs, produces materials that inherently exhibit many other desirable characteristics. Au NPs are gradually attracting a great deal of attention for their use in future technologies, including catalysis, optical materials, electronic devices, biosensors, drug carriers, and high contrast cell imaging [25–30]. Au NPs have been widely used in analytical procedures because of their size-dependent electrical properties, high electrocatalytic activity, and functional compatibility with molecules and polymers [4]. Besides, biomolecules containing thiol (SH) or amino (NH_2) groups can be adsorbed spontaneously onto gold surfaces to generate well-organized, self-assembled monolayers [25]. Although Au NPs provide a large surface area to interact with column surface and analyte, very little research has been devoted to understanding their impact on separation science. Although the application of Au NPs is still in its infancy, previous studies have demonstrated their potential as multifunctional components to improve the chemical stability, analytical selectivity, and separation efficiency. This review is focused on various characterizations of Au NPs by using chromatography- and electrophoresis-based methods such as size exclusion chromatography (SEC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and micellar electrokinetic chromatography (MEKC). In addition, the applications of Au NPs in particular separation and preconcentration methods, including the open-tubular capillary electrochromatography (OTCEC), microchip, gas chromatography (GC), HPLC and CE, are classified based on their modes of analytical operation.

Characterization and analysis of Au NPs

In any application of Au NPs, it is of particular importance to determine their physical and chemical properties. The minimization of gold from condensed matter to nanoscale particles produces materials that demonstrate versatile properties of optical surface plasma absorption, emission illumination at quantum size, optical enhanced Raman spectroscopy, melting point reduction, electrical Coulomb blockade effect, and single electron transistor behavior. In

this section, several analytical methods which employ chromatography and electrophoresis for characterization on Au NPs are introduced.

Analysis of Au NPs using chromatographic techniques

Chromatographic techniques, traditionally used to separate small molecules, biomolecules, and polymers according to their size, chemical interaction, and/or physical interaction, have been recently applied to analyze the cluster size distributions of Au NPs [31]. Currently, there are many chromatographic methods available for the separation of Au NPs [32], such as SEC [13, 31, 33–36], HPLC [37–41], and ion-exchange chromatography [42].

SEC has been used to analyze Au NPs in aqueous solutions [13, 34]. Accurate calibration of commercially available Au NPs standards by electron microscopy has shown that SEC analysis can be a stand-alone technique to characterize nanometer-sized spherical Au NPs ranging in size from 3 to 20 nm [35]. SEC separations are generally accomplished by the selection of a suitable stationary phase that has an appropriate pore size, along with the use of appropriate eluent. However, irreversible adsorption of NPs to stationary phase is still a challenging issue facing the use of chromatographic techniques for NPs separation. Both the high surface area of stationary phase and the high surface activity of NPs increase the problem of irreversible sorption. Surfactant is known to associate with stationary phase, subsequently affecting the retention behavior of the analyte [43]. The use of surfactant above critical micellar concentration in SEC was previously developed for the separation of inorganic ions and small molecules [44]. Elution behavior of SEC with micelle generally follows the three-phase model of micellar liquid chromatography developed by Armstrong et al. [45]. One of the available surfactants, sodium dodecyl sulfate, as mobile phase additive prevents the above-mentioned problem of adsorbing and coagulating during the size determination of Au NPs, thereby facilitating their size-dependent analysis by the SEC approach [13, 31, 33, 34].

HPLC is also a popular column tool used in chromatography practice. HPLC has proven useful for separating, characterizing, and measuring the properties of a broad range of colloidal materials because of the great utility, excellent performance, long-term stability, and wide variety of HPLC set-ups available. With the aim of separating and analyzing water-insoluble Au NPs in which the NPs were protected by a non-polar surface-coating layer (e.g., alkanethiolate-modified Au NPs), Murray et al. proposed reversed-phase HPLC [38–41] in combination with diode array detector (DAD) to determine the relative monodispersity of Au NPs. They also developed cyclic voltammetric detection to monitor ultrasmall Au NPs

(core diameters less than 2 nm) separated by an HPLC-based technique [37].

Analysis of Au NPs using electrophoresis

Because the surface of water-soluble NPs bears the charges during dispersion in aqueous state or migration over an electric field, the electrophoretic method is a good alternative candidate for concentrating and analyzing NPs [32]. Electrophoretic analysis of micrometer- and nanometer-sized particles by gel electrophoresis [46, 47], continuous free-flow electrophoresis [48], capillary zone electrophoresis [49–55], and capillary gel electrophoresis [56] have all been reported. Whetten and co-workers have demonstrated an Au cluster system generated by the reduction of Au and polymer conjugate. Such an inorganic-organic hybrid material, composed of an Au core and a glutathione adsorbate layer, can be efficiently separated by polyacrylamide gel electrophoresis (PAGE) to yield a series of distinct compounds only in the range of 20–80 atoms [47]. However, several adverse effects may hinder the further use of PAGE in preparative electrophoresis. Firstly, the pore size of polyacrylamide gel (PAG) is typically about 3–5 nm [57], whereas the purification of larger particles needs bigger pore matrices. Secondly, PAG synthesized by free radical polymerization exhibits strong heterogeneity in pore sizes [58]. Finally, it is not easy to scale up because of severe shrinkage of PAG after polymerization. Therefore, Xu et al. improved the efficiency of preparative electrophoresis to allow separation of Au NPs based on size and shape by using agarose gels [46]. The technique was first demonstrated by size separation of 5, 15, and 20 nm spherical Au nanoclusters; and the purification of a crude nanorods suspension was further separated into three individual components (i.e., pure long nanorods, nanoplates, and spheres).

CE has recently emerged as one of the most powerful separation techniques [59]; it can be used to separate a variety of differently sized materials, including inorganic Au and Ag NPs [50, 53–55]. The separation mechanism relies upon these particulate nanoparticles having surface charges and, therefore, their separation behavior is similar to that exhibited by charged molecular species during CE. Differently sized particles display specific mobilities in a capillary column under the influence of an external applied voltage because they have different charge-to-size ratios. Thus, the use of high electric fields and high ionic strength electrolyte buffers can lead to the pronounced relaxation effect [60–64]. The relaxation effect is described by a dimensionless parameter κa, where a is the particle radius and κ^{-1} is the thickness of the electric double layer (EDL). Deformation of the EDL around the particle—the degree of which differs depending on the size of the particle and

buffer ionic strength—results in size-dependent electrophoretic migration behavior of these particles; as a result, CE can be used to separate these particles based on their sizes [62].

Although CE is a powerful technique that is simple to perform, involves low sample consumption, has high efficiency and resolution, and provides rapid results, the benefits provided by the high number of theoretical plates obtained with CE can be overshadowed by the low sensitivity of UV detection systems [65]. Because of the small dimensions of CE capillaries [typical inside diameters (I.D.) and lengths are 25–100 μm and 35–80 cm, respectively], only very small amounts of sample can be analyzed. To separate sample species and to increase the amount of sample size that can be loaded into the column without degrading the separation, several online sample preparation techniques have been developed for various electrophoretic modes [66–68]. In particular, one of the methods enhancing detection sensitivity of CE, named reversed electrode polarity stacking mode (REPSM), was proposed by Terabe and et al. [69, 70]. The REPSM of CE involves the introduction of the sample into the capillary hydrodynamically, applying a stacking voltage at negative polarity to preconcentrate the analytes at the interface between the sample zone and the background electrolyte, and then pumping the sample matrix from the capillary under electroosmotic flow (EOF). In comparison with the traditional CE, the REPSM technique of CE is an efficient and reliable method that allows the selected analytes of low concentration to be separated and detected effectively.

The MEKC-based online preconcentration strategy, the so-called REPSM for CE, generally provides excellent detectability. Since Terabe and co-workers reported their first work on MEKC [71], it has become a widely accepted separation mode for CE [72, 73]. To overcome the limitation of NPs analysis by CE, we first proposed the feasible means of using MEKC in conjunction with the REPSM for characterizing the sizes of Au NPs [74] and separating Au/Ag core/shell NPs [75] obtained through seed-assisted synthesis. We prepare a sample mixture containing four differently sized Au NPs to find out the appropriate MEKC operating conditions and to investigate whether a linear correlation exists between electrophoretic mobility and Au NPs diameter [74]. The electropherograms reveal that the differently sized Au NPs had different migration times (Fig. 1, left). Additionally, a good linear relationship ($R^2 = 0.99$) does exist between electrophoretic mobility and Au NPs size (Fig. 1, right). The online enhancement improved the detection sensitivity toward the Au NPs by up to 260-fold. An attractive feature of more recent research uses the DAD system to online record the optical spectra for various sizes of Au NPs after the MEKC separation [76]. Thus, using this new approach, each size of Au NPs sample with low concentration can be easily separated through MEKC within a short analysis time (less than 4 min) while simultaneously obtaining their size-dependent optical properties; such analyses are impossible to perform with traditional dynamic light scattering and modern electron beam-based microscopy techniques for the characterization of Au NPs.

Application of Au NPs for separation and preconcentration

Self-assembly

Self-assembly is the fundamental principle which generates structural organization on all scales from molecules to galaxies. Self-assembly is defined as reversible processes in which preexisting parts or disordered components of a preexisting system form structures of patterns. Surface self-assembly of molecules onto Au NPs can be classified as either a static or dynamic process. During the utilization of Au NPs for separation or preconcentration, molecular assemblies are formed spontaneously by the immersion of an appropriate size of NPs in a solution. Self-assembly techniques for the NPs applications utilize physisorption (e.g., electrostatic adsorption of charged molecules from NPs colloidal solution) or chemisorption (e.g., covalent bond formation between an organic molecule group and NPs) interaction.

Surface modification with functional groups of molecules is a key procedure to prevent the prepared NPs from aggregating and to control the particle size, and also provides extra selectivity when the NPs are used as separation media. The fabrication of structures by means of the self-assembly strategy has attracted much attention because of the simplicity and flexibility of this approach [77]. A couple of methods have been proposed for the self-assembly of Au NPs onto surfaces functionalized with SH and NH_2 groups [78]. These functionalized Au NPs have served as stationary phase materials for various separation and preconcentration systems, and the most commonly employed techniques in Table 1 are solid-phase extraction (SPE), GC, HPLC, OTCEC, microchip, and CE.

Solid-phase extraction

SPE is one of the most widely used tools for the preconcentration and clean-up of analytical samples, for the purification of various chemicals, and for the removal of toxic or valuable substances from aqueous solutions [79]. SPE has gained wide acceptance because of its ease of operation, high recoveries, reproducibility, and commercial availability of appropriate sorbents. The applications of Au
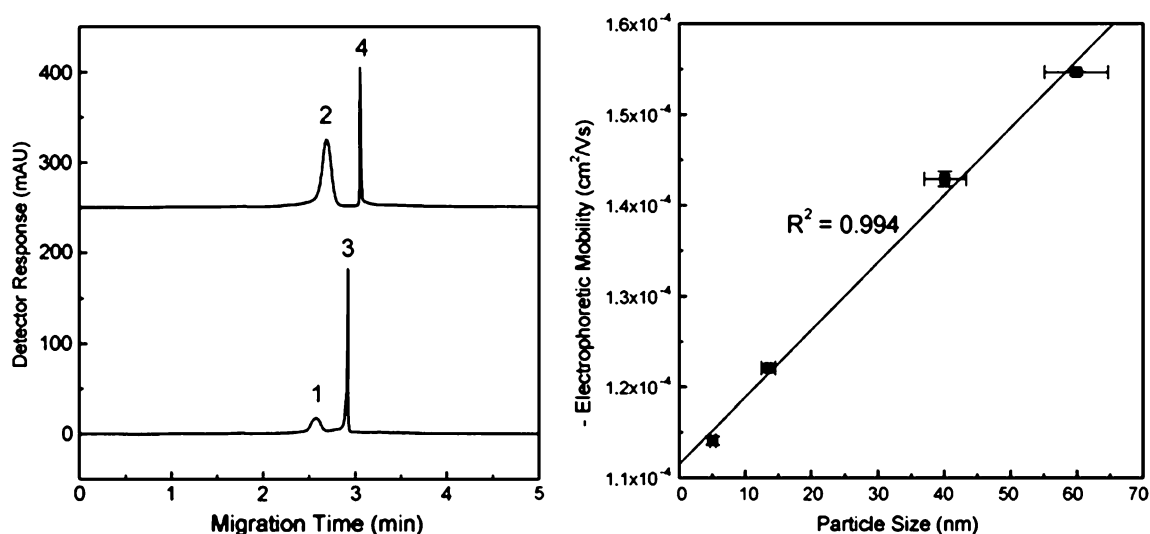
Fig. 1 *Left* Electropherograms of Au NPs (*1* 5.3-, *2* 13.1-, *3* 40.1-, and *4* 59.9-nm-diameter particles). *Right* Calibration curve displaying the electrophoretic mobility as a function of the diameter of the Au NPs. (reprinted with permission from Ref. [74])

NPs in SPE for HPLC [80–82], GC [83], and CE [84] analysis have been reported.

A novel alternative approach using the so-called solid-phase nanoextraction (SPNE) for the preconcentration of polycyclic aromatic hydrocarbons (PAHs) from drinking water has been proposed by Wang et al. [80, 82]. PAHs are a complex class of condensed multi-ring benzenoid compounds originating from a wide variety of natural and anthropogenic sources. Generally, 500 µL of contaminated water sample is mixed with 950 µL of a solution of 20 nm Au NPs for pollutants extraction. Next, centrifugation takes place, supernatant is discarded, and the adsorbed analytes are released from the Au NPs by adding 2 µL of 1-pentanethiol and 48 µL of *n*-octane. The resulting solution is analyzed either by HPLC [80] or laser-excited time-resolved Shpol'skii spectroscopy [82]. The optimization of experimental parameters led to a novel method with excellent analytical figures of merit, high recovery, and short analysis time. Moreover, as the entire extraction procedure consumes less than 100 µL of organic solvents per sample, the method can be considered an environmentally friendly approach for routine analysis of numerous samples. The small volume of extracting solution makes SPNE a relatively inexpensive extraction approach in comparison with other micro and conventional SPE procedures. In particular, preconcentration of aromatic analytes on Au NPs was successfully used to improve the analytical recovery during the analysis of monohydroxy-PAHs (OH-PAHs) in urine samples [81]. In this case, a typical SPE-HPLC procedure is profitably combined with SPNE. The reported data from human urine samples provide a general prospective for the excellent potential of the new approach for the analysis of OH-PAHs.

Recently, we have developed an SPE-sweeping-MEKC methodology for the successful preconcentration of neutral steroid analytes [84] such as testosterone, progesterone, and testosterone propionate from urine samples. The sorbent is fabricated from two alkanethiol self-assembly processes: one to deposit the Au NPs onto a 3-aminopropyltrimethoxysilane (APTMS)-modified silica gel, and the other to functionalize the surfaces of the Au NPs. The use of Au NPs layer-by-layer (LBL)-coated SPE materials not only concentrated the neutral steroids effectively through hydrophobic interactions with the Au NPs-capped silica gel but also removed the interfering signals from urinary proteins through their interactions with residual Au metal surfaces. Modification of Au NPs-coated silica SPE material in combination with the optimized sweeping MEKC allows for very efficient preconcentration and sample clean-up, and the stacking of the analytes results in the 10^2- to 10^3-fold sensitivity enhancement for the analysis of steroids.

Chromatographic separations

Gas chromatography

The first application of Au NPs in capillary GC was described by Gross et al. in 2003 [85]. They prepared monolayer-protected Au NPs (MPN) by covalent immobilization of dodecanethiol onto the Au surface. An MPN film was deposited in a 2-m-long and 530-µm-I.D. deactivated silica capillary by using gravity to flow the solution containing the MPN material through the capillary. By scanning electron microscope (SEM) analysis, the average film thickness was estimated to be 60.7 nm and uniformly

Table 1 Summary of applications for Au NPs-assisted techniques in separation and preconcentration

Analytical technique	Principle	Description	Analytes	Author/group, year (reference)
SPE	Strong affinity between Au NPs and PAHs	Based on HPLC technique, solid-phase nanoextraction and centrifugation	PAHs in urine samples	Campiglia's group, 2008 [80], 2009 [81, 82]
	Alkanethiol-modified Au NPs coated on silica gel	Based on GC technique, LBL assembly for different thicknesses of Au NPs layers	Steroids (progesterone and testosterone propionate)	Liu, 2008 [83]
	Au NPs LBL-coated on silica gel	Based on CE technique, SPE-sweeping-MEKC method	Steroids (testosterone, progesterone, and testosterone propionate) in urine samples	Liu, 2008 [84]
GC	Dodecanethiol MPN modified capillary	The first application of Au NPs in OTCGC, capillary = 2 m, 530-μm I.D.	Four compound classes (alkanes, alcohols, aromatics, and ketones), eight-component mixture separations	Gross et al., 2003 [85]
	Dodecanethiol MPN modified microchannel	Microchannel = 1.3-m long, square (100 μm×100 μm), high-speed microfabricated GC (within 2 s)	Four compound classes (alkanes, alcohols, ketones, and aromatics), seven-component mixture separations	Gross et al., 2004 [86, 87]
	ODA-Au NPs modified capillary (electrostatic and hydrophobic interactions)	Capillary = 1.6 m, 100-μm I.D., high separation efficiency with run-to-run and column-to-column reproducibility	Nine compounds (ethanol, benzene, 1-butanol, chlorobenzene, 1-pentanol, anisole, phenol, methyl benzoate, benzyl alcohol)	Qu et al., 2008 [88]
HPLC	Immobilization of n-octadecanethiol-modified Au NPs on capillary	The first application of Au particles in capillary HPLC, capillary = 100-μm I.D., Au particles diameter = 3.5 μm	Oganic compounds (aniline, p-toluidine, toluene, ethylbenzene, p-hydroquinone, benzoic acid, and 2-nitrophenol)	Qu et al., 2008 [89]
	Immobilization of n-octadecanethiol-modified Au-coated polystyrene particles on capillary	High affinities for phenanthrene and anthracene in the C18-Au column (unique separation behavior), drawback of low separation efficiency and heavy peak tailing	Aromatic compounds (benzene, naphthalene, phenanthrene, and anthracene)	Kobayashi et al., 2006 [90]
	Immobilization of BSA-modified Au NPs on capillary	Self-assembly strategy of proteins, enantioselective recognition	Dansyl-norvaline	Liu et al., 2003 [91]
OTCEC	Dodecanethiol-Au NPs modified capillary by APTMS/MPTMS	The first application of Au NPs in OTCEC, reproducible retention, and characteristic reversed-phase behavior	Neutral compounds (thiourea, benzophenone, biphenyl, and pyrethroid pesticides)	Glenonn's group, 2003 [92]
	Etching process on capillary, prior to assembly of the dodecanethiol-modified Au NPs	Increase the surface area with higher phase ratio for better separation efficiency	Neutral compounds (thiourea, naphthalene, and biphenyl) and PAHs	Glenonn's group, 2005 [97]
	Sol-gel approach on capillary, prior to assembly of the dodecanethiol-modified Au NPs	Large surface stationary phases with high stability, mass loadability, and separation efficiency	Drug substances (propiophenone, benzoin, and warfarin) and PAHs	Glenonn's group, 2005 [98]
	Repeated modification of the alkanethiol-modified Au NPs by 1,9-nonanedithiol	LBL assembly, reproducible retention times, and characteristic reversed-phase behavior	Steroid drugs (testosterone, progesterone, and testosterone propionate)	Liu et al., 2005 [99]
	ODA-Au NPs modified capillary (electrostatic and hydrophobic interactions)	Reproducible retention times and high separation efficiency	Neutral compounds (thiourea, naphthalene, and biphenyl) and steroid drugs (testosterone, progesterone, and testosterone propionate)	Qu et al., 2008 [101]
Microchip	Au NPs-modified PDMS microchip	LBL assembly (PDMS-polyelectrolyte-Au NPs), enhanced separation efficiency	Neurotransmitters (DA and EP)	Wang et al., 2006 [102]
	Protein-modified PDMS microchip	LBL assembly (PDMS-polyelectrolyte-Au NPs-protein), enhanced sensitivity and EOF stability	Neurotransmitters (DA and EP) and environmental pollutants (p-phenylenediamine, 4-aminophenol, and hydroquinone)	Wang et al., 2006 [103]

Table 1 (continued)

Analytical technique	Principle	Description	Analytes	Author/group, year (reference)
	EOF-switchable PDMS microchip	LBL assembly (PDMS-PDDAC-Au NPs-cysteine), switched EOF by varying the pH of running buffer	Neurotransmitters (DA and EP) and amino acids (arginine and histidine)	Wang et al., 2007 [104]
CE	DPPAC-Au NPs modified capillary	The first application of Au NPs in CE, improved selectivity and control the EOF	*p*-Phenylendiamine, *p*-aminophenol, *p*-anisidine, and *p*-toluidine	Grushka and co-workers, 2001 [109]
	DPPAC-Au NPs modified microchannel	The first application of Au NPs in microchip CE system, selectivity improvement	*p*-Aminophenol, *o*-aminophenol, and *m*-aminophenol	Grushka and co-workers, 2001 [110]
	Au NPs/polymer composites	High-resolution DNA separations with low viscosity PEO solutions containing Au NPs	dsDNA fragments	Chang's group, 2003 [112]
	Au NPs/polymer composites	NFCE, successful separation of 8.27–48.5 kbp DNA markers	Long dsDNA fragments	Chang's group, 2004 [113]
	Au NPs/polymer composites	LBL assembly (PMMA-PVP-PEO-Au NPs), microchip CE	8–2,176 bp dsDNA fragments	Chang's group, 2003 [114]
	Surfactant-capped Au NPs	NFCE, peak efficiency and reproducibility improvements	Acidic/basic proteins and biological samples (saliva, red blood cells, and plasma)	Tseng's group, 2006 [122]
	Combination with NFCE and centrifugal ultrafiltration	Sensitivity improvement (314-fold)	Acidic/basic proteins and egg white proteins	Tseng's group, 2008 [125]
MS	Cationic/anionic functional groups-modified Au NPs as extraction agents	Analysis of Au NPs/peptide composites by MALDI-MS after centrifugation	Negatively/positively charged peptides and digested myoglobin	Vanderpuije et al., 2006 [126]

APTMS 3-aminopropyltrimethoxysilane, *Au NPPs* Au NPs/polymer composites, *Au NPs* gold nanoparticles, *BSA* bovine serum albumin, *CE* capillary electrophoresis, *C18* *n*-octadecanethiol, *DA* dopamine, *DNA* deoxyribonucleic acid, *dsDNA* double-stranded deoxyribonucleic acid, *EOF* electroosmotic flow, *EP* epinephrine, *GC* gas chromatography, *HPLC* high-performance liquid chromatography, *LBL* layer-by-layer, *MALDI-MS* matrix-assisted laser desorption/ionization-mass spectrometry, *MEKC* micellar electrokinetic chromatography, *MPN* monolayer-protected Au NPs, *MPTMS* 3-mercaptopropyltrimethoxysilane, *MS* mass spectrometry, *NFCE* nanoparticle-filled capillary electrophoresis, *ODA* octadecylamine, *OTCEC* open-tubular capillary electrochromatography, *OTCGC* open-tubular capillary gas chromatography, *PAHs* polycyclic aromatic hydrocarbons, *PDDAC* poly(diallyldimethylammonium chloride), *PDMS* polydimethylsiloxane, *PEO* poly(ethylene oxide), *PMMA* poly(methyl methacrylate), *PVP* poly(vinyl pyrrolidone), *SPE* solid-phase extraction

distributed over the majority of the inner capillary surface. As to the issue of self-assembled Au NPs for GC application, Gross et al. studied the retention behavior of the dodeca-nethiol MPN column toward four compound classes (i.e., alkanes, alcohols, aromatics, ketones) and compared it with a commercial AT-1 column in which the coated film thickness was 100 nm. An eight-component mixture was separated in MPN by using both isothermal and temperature-programming methods in comparison with an isothermal separation with the AT-1 stationary phase (Fig. 2). Furthermore, more impressive GC separations were afforded by using a dodecanethiol MPN stationary phase within a microchannel environment with a 1.3-m-long, square (100 μm×100 μm) capillary column as a model for high-speed microfabricated GC [86, 87]. The thickness of the MPN phase along the capillary walls was determined to be 15 nm by SEM analysis. When the film depth was very uniform, the chromatographic performance was very high and even mixtures of seven analytes were well separated

within 2 s [87]. In addition, application of the square capillary MPN column as the second column of a comprehensive 2D GC system was also explored carefully.

We have found that the number of layers of Au NPs coated on the silica gel affect the preconcentration performance for steroids [83]. We describe the efficacy of preconcentration prior to GC analysis by using a sorbent constructed from silica gel capped with self-assembled *n*-octadecanethiol and Au NPs. Figure 3 (left) displays SEM images of glass substrates coated with two different thicknesses of Au NPs layers. When using a six-layer Au NPs-coated silica gel sample, we obtain the highest preconcentration efficiency for neutral steroid drugs that is improved by a remarkable 170-fold relative to that of the regular analysis processes (Fig. 3, right). The number of layers on the silica gel dramatically influences the loading density of hydrophobic moieties on the silica gel; therefore, adjusting the number of layers is a suitable means of controlling the degree of drug molecule preconcentration.
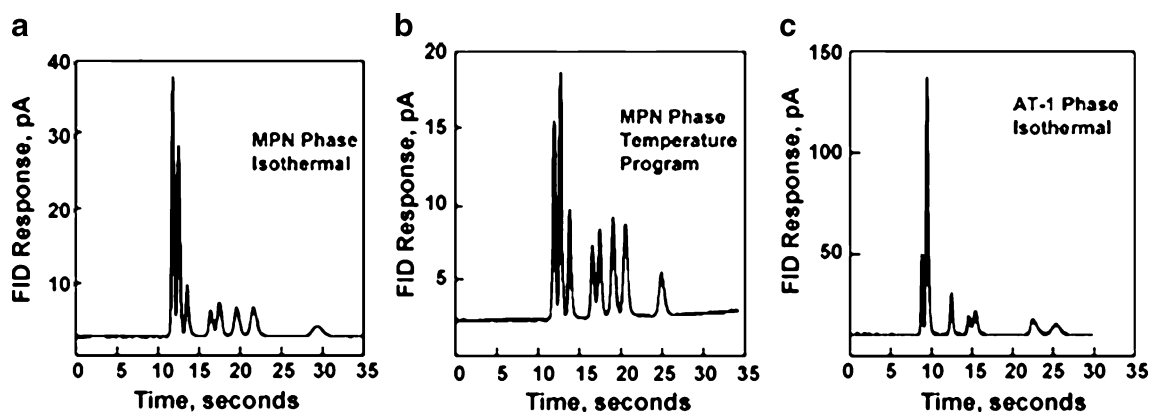
Fig. 2 Eight-component mixture separations using the dodecanethiol MPN column: **a** isothermal (50 °C) and **b** temperature-programmed (40–80 °C at 70 °C/min). **c** Isothermal separation at 50 °C on an AT-1 commercial column. Elution order: ethanol, benzene, 1-butanol, chlorobenzene, 3-heptanone, anisole, 3-octanone, and decane (reprinted with permission from Ref. [85])

Additionally, octadecylamine (ODA)-capped Au NPs prepared by Qu et al. were successfully applied in GC [88]. The ODA-Au NPs can be adsorbed tightly onto the inner surface of fused silica capillary column via electrostatic interaction and can enhance the van der Waals interactions between Au NPs and capillary wall. There was no perceptible degradation in the ODA-Au NPs separation after ca. 1,900 sample runs. Besides, good separation efficiency with respect to the retention time was achieved with run-to-run, day-to-day, and column-to-column repro-ducibility, indicating the structural and functional integrity of the ODA-Au NPs stationary phase during GC separation.
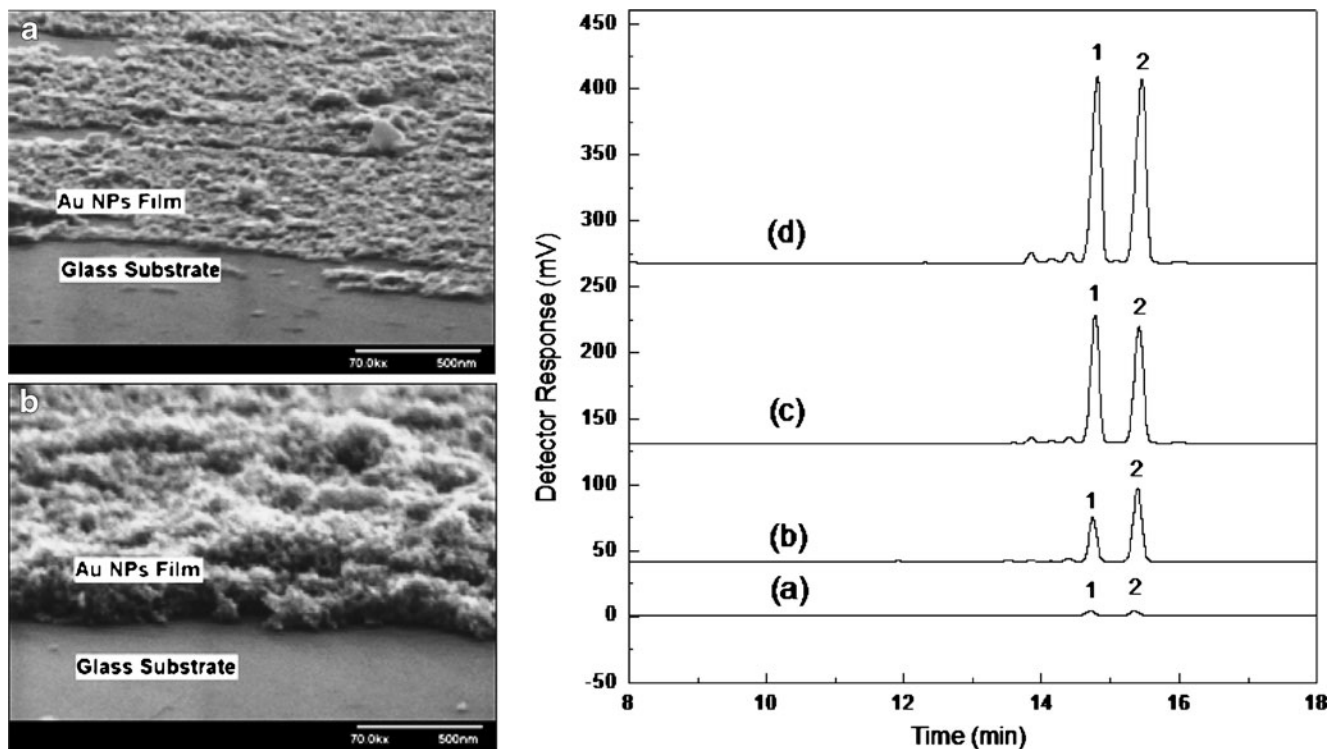

Fig. 3 *Left* SEM images of glass slides coated with (**a**) a monolayer and (**b**) six layers of Au NPs. *Right* Gas chromatograms obtained after preconcentration of the tested steroids in the absence of silica gel (*a*) and using monolayer (*b*), three-layer (*c*), and six-layer (*d*) Au NP-coated silica gel samples. The steroid solution [100 mL, progesterone and testosterone propionate (5 mg L^{-1}) in 5% ACN/95% deionized water (v/v)] was passed through each silica gel sample and then the retained steroid was extracted from the silica gel using pure ACN (0.5 mL); the resulting solutions were characterized using GC. Samples: *1* progesterone; *2* testosterone propionate (reprinted with permission from Ref. [83])

High-performance liquid chromatography

Qu and co-workers also synthesized the organic-inorganic hybrid of *n*-octadecanethiol (C18-Au NPs) and packed the material into a 100-μm-I.D. capillary column to conduct capillary HPLC (μHPLC) experiments [89]. They considered that Au microspheres have great potential to become promising substitutes for silica-based stationary phases for μHPLC due to their stability at high pH, enough rigidity, and ease of chemical modification. Their results showed that the new packing material behaves basically as a reversed phase. The average diameter of these Au microspheres was determined to be about 3.5 μm by SEM (Fig. 4, left). Chemical stability of the column was excellent even at alkaline pH 12 for at least 140 h; mechanical stability was also strong enough to withstand pressure up to 52 MPa. The column allows separation of neutral, acidic, and basic samples and provides symmetrical peaks with high efficiency, e.g., as illustrated by the separation of a mixture of small organic compounds in Fig. 4 (right). The prospects for such organic-inorganic hybrid materials are promising for biomolecule separation. In 2006, Kobayashi et al. reported a capillary column for HPLC packed with Au-coated polystyrene particles (C18-Au column), which is modified by self-assembled thiol compounds to create the stationary phase [90]. The difference in selectivity for aromatic compounds between thiol-modified Au-coated polystyrene particles and the ordinary octadodecyl-modified silica gels was investigated. In particular, the high affinities of phenanthrene and anthracene toward the thiol compounds on the Au surfaces revealed their unique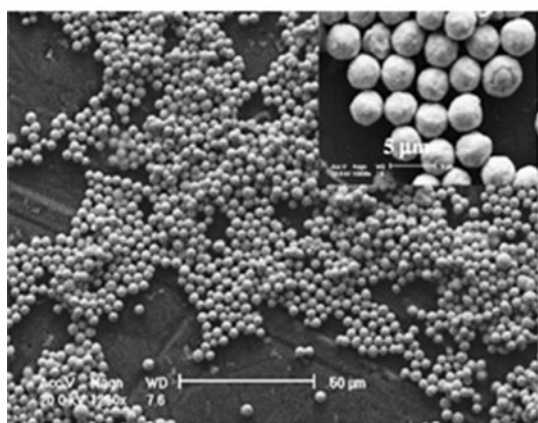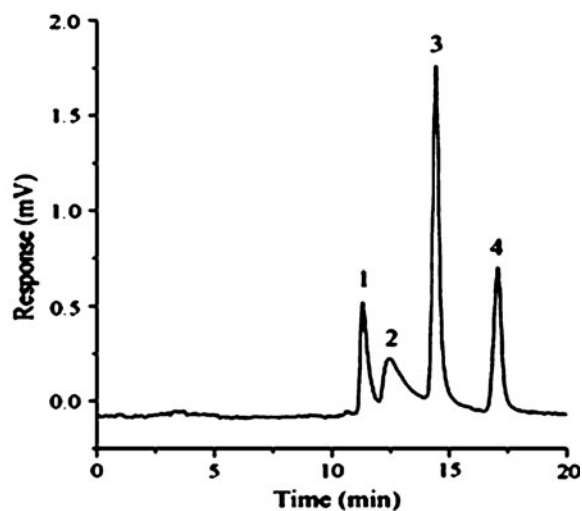 elution behavior relative to that obtained using conventional columns. Thus, specific retention in thiol-modified Au columns can be used to achieve unique separation behavior. In our previous study, bovine serum albumin (BSA)-modified Au NPs were used as an HPLC stationary phase for enantioselective recognition of dansyl-norvaline [91]. The proposed HPLC technique offers the feasibility to investigate the interactions between modified molecules and solutes in a rather simple way. Thus, this approach may be applied to self-assembly strategies for other biomolecules other than BSA.

Electrophoretic separations

Open-tubular capillary electrochromatography

An alternative mode of capillary electrochromatography (CEC) is OTCEC, in which the stationary phase is coated on the inner wall of the open capillary column. When compared to packed-column CEC, the main advantages of OTCEC are its high efficiency, simple instrumental handling, and short conditioning times. The first covalent immobilization of Au NPs on the prederivatized fused-silica capillaries and their application in OTCEC was published by Glennon and co-workers in 2003 [92]. They first functionalized the Au NPs surface with dodecanethiol. In order to make the surface hydrophobic, these particles were covalently anchored with the prederivatized APTMS or 3-mercaptopropyltrimethoxysilane (MPTMS) capillary (Fig. 5, left). The electrochromatographic properties of the Au NPs-modified capillaries were investigated by using a reversed-phase test mixture of neutral compounds, thiourea (EOF marker), benzophenone, biphenyl, and pyrethroid

Fig. 4 *Left* SEM image of *n*-octadecanethiol-modified Au microspheres; *inset* high-resolution image. *Right* Chromatogram of a mixture of basic and neutral compounds. Column: 45 cm (effective length 25 cm)×100-μm-I.D. capillary packed with 3.5 μm Au microspheres modified with *n*-octadecanethiol. Peak identification: *1* aniline, *2* *p*-toluidine, *3* toluene, *4* ethylbenzene (reprinted with permission from Ref. [89])
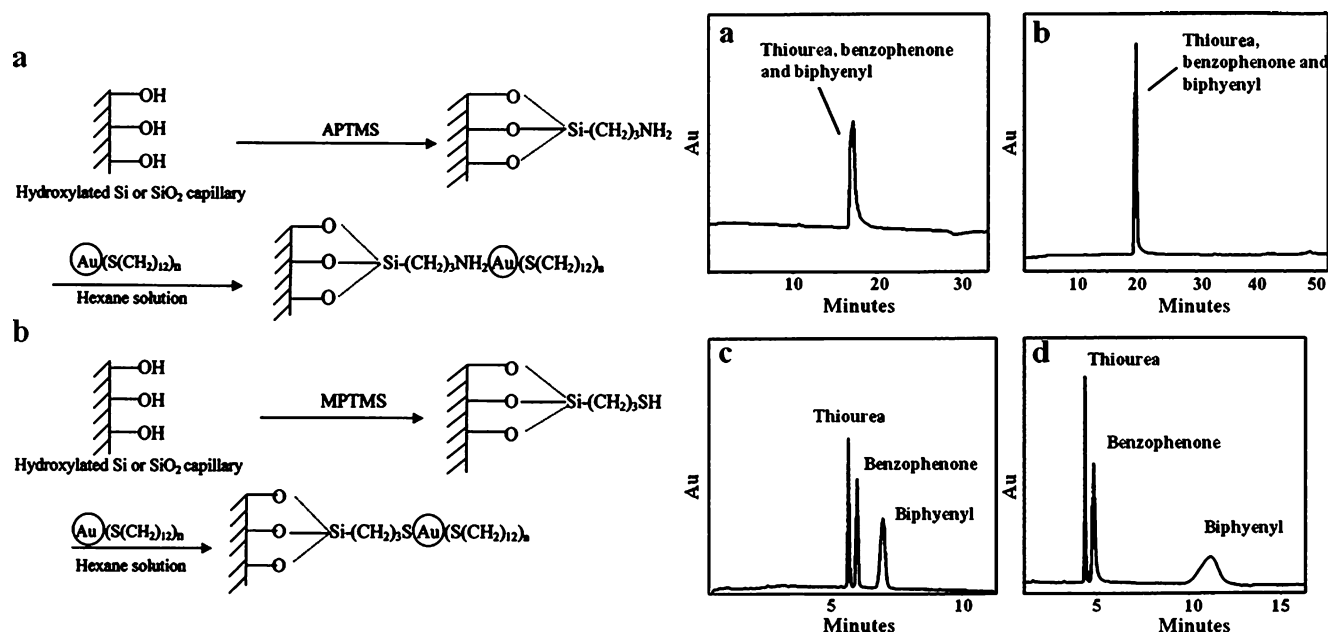
Fig. 5 *Left* Reaction steps involving alkylthiol Au NPs and fused-silica capillary prederivatized with **a** APTMS and **b** MPTMS. *Right* Electrochromatograms of test-mixture separations on **a** an MPTMS-coated capillary (45% MeOH), **b** an APTMS-coated capillary (45% MeOH), **c** an Au MPTMS-coated capillary (70% MeOH), and **d** an Au APTMS-coated capillary (35% MeOH) (reprinted with permission from Ref. [92])

pesticides. Glennon et al. reported that the dodecanethiol-capped Au NPs capillary columns are easier to produce and operate relative to packed capillary columns (Fig. 5, right).

The OTCEC concept is based on the Au NPs-coated capillaries and demonstrates many positive aspects. However, the phase ratio and sample capacity in an OTCEC format are relatively poor due to the limited amount of stationary phase coating [93]. In order to overcome the above limitation, Glenonn's group follows the approach from Pesek and co-workers [94–96] and prepares Au NPs-coated capillaries by first etching the capillaries with ammonium hydrogen difluoride, then prederivatizing them with MPTMS, and finally immobilizing the dodecanethiol Au NPs [97]. The etching process is very efficient and increases the surface area by an amplification factor up to 10^3 for the inner wall of the capillaries and, consequently, enhances interaction between the analytes and the immobilized stationary phase. An alternative approach rendering an increased phase ratio is provided by the sol-gel technique [98], which provides large surface stationary phases with high stability, mass loadability, and separation efficiency. MPTMS is also selected as the sol-gel precursor to develop a sol-gel layer on the inner wall of the capillary, prior to assembly of the dodecanethiol-modified Au NPs. The sol-gel Au NPs capillary exhibits good electrochromatographic behavior, and confirms that the dodecanethiol Au NPs bound on the sol-gel-based inner layer of the fused-silica capillary provide sufficient solute-bonded phase interac-

tions for OTCEC with reproducible retention behavior. Both the etching and sol-gel approaches demonstrate capillaries with higher phase ratio, thus allowing for better separation efficiency and selectivity for important analytes, such as polyaromatic hydrocarbons and several drug substances (e.g., propiophenone, benzoin, and warfarin).

We have also investigated the self-assembly strategy of Au NPs onto OTCEC columns to enhance the phase ratio [99]. To increase the loading density of Au NPs on the surface of the capillary, LBL assembly is a simple preparation technique for the structural organization of a large variety of Au NPs into multilayer films [99, 100]. The covalently immobilized Au NPs are either modified with several alkanethiols of variable lengths (1-hexanethiol, 1-octanethiol, 1-dodecanethiol, and 1-octadecanethiol) to create a hydrophobic monolayer film or LBL technology based on the (several times) repeated modification of the Au NPs surface with 1,9-nonanedithiol and Au NPs are employed to provide a multilayered film on the inner capillary wall (Fig. 6, left). The effect of Au NPs layers was studied and retention of the hydrophobic analytes significantly increased with the number of layers created in the capillary; the four-layer film was considered to be the optimal arrangement (Fig. 6, right). Through the self-assembly onto Au NPs-coated capillaries, three neutral steroid drugs, testosterone, progesterone, and testosterone propionate, were successfully separated on OTCEC columns prepared by the LBL process. In addition, this system
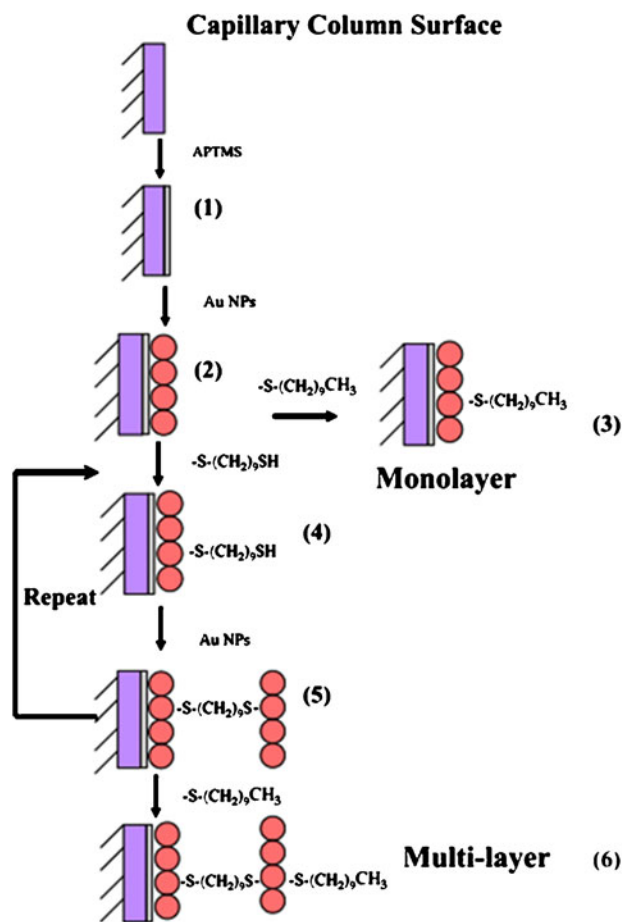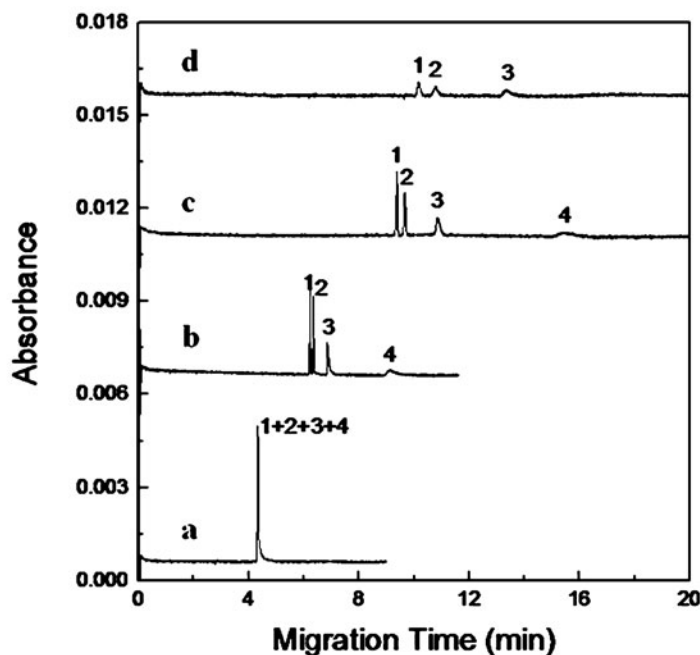
Fig. 6 *Left* Schematic representation of the process used to coat capillary columns with films of self-assembled alkanethiols on monolayers and multilayers of Au NPs. *Right* Electrochromatograms obtained from the CEC separations of a test mixture of steroid drugs: *a* one, *b* two, *c* four, and *d* seven layers. Samples: *1* EOF; *2* testosterone; *3* progesterone, and *4* testosterone propionate (reprinted with permission from Ref. [99])

provides reproducible retention times and characteristic reversed-phase behavior.

ODA-capped Au NPs prepared by Qu et al. are successfully applied not only in GC [88] but also in OTCEC [101]. Recently, ODA-Au NPs have also been prepared and tested for OTCEC separation of thiourea, naphthalene, biphenyl, and several pharmaceutical steroid drugs [101]. Qu and co-workers investigated various experimental parameters such as buffer pH, buffer concentration, and percentage of organic modifier in the mobile phase. All of the results indicated that the modified Au NPs could act as a stable stationary phase for OTCEC as well as for bioanalysis.

Microchip

Successful separation of the neurotransmitters dopamine (DA) and epinephrine (EP) on Au NPs-modified poly-dimethylsiloxane (PDMS) microchips prepared by the electrostatic LBL assembly technique was demonstrated by Wang et al. [102]. The authors investigated four

polyelectrolytes, linear polyethylenimine (LPEI), poly (diallyldimethylammonium chloride) (PDDAC), poly (allylamine) hydrochloride, and chitosan. The microchip surface is coated with suitable polyelectrolyte and formed a cationic surface. The interactions between Au NPs and polyelectrolyte-coated channel surface alter the electroosmotic velocity and also changed its EOF direction. The modification procedure using LPEI is depicted in Fig. 7 (left). LPEI or PDDAC modified channels provide more separation efficiency with more symmetrical peaks for DA and EP (Fig. 7, right). The results show the resolution and the analysis times for native and coated microchips were greatly enhanced from 0.62 to 1.14 and from 55 s to 100 s, respectively. Moreover, protein-coated PDMS microfluidic chips are also constructed by the LBL technology [103]. Separation efficiencies are significantly improved on the proteins and NPs-coated microdevices in comparison with microchip without NPs. It is clearly evident that Au NPs could greatly enhance the sensitivity and EOF stability. In addition, an EOF-switchable PDMS chip modified by Au NPs with cysteine has been developed

Fig. 7 *Left* The PDMS/PDMS microchip modification procedures. *Right* Electropherograms showing the separations of 400 μM DA and 400 μM EP: **a** in native PDMS/PDMS microfluidic chip; **b** in coated PDMS/PDMS microfluidic chip. Conditions: 30 mM PBS (pH 7.0) as running buffer; sample injection at +800 V for 4 s; separation voltage +800 V. The signals were recorded after 20 s in coated PDMS/PDMS microchips (reprinted with permission from Ref. [102])
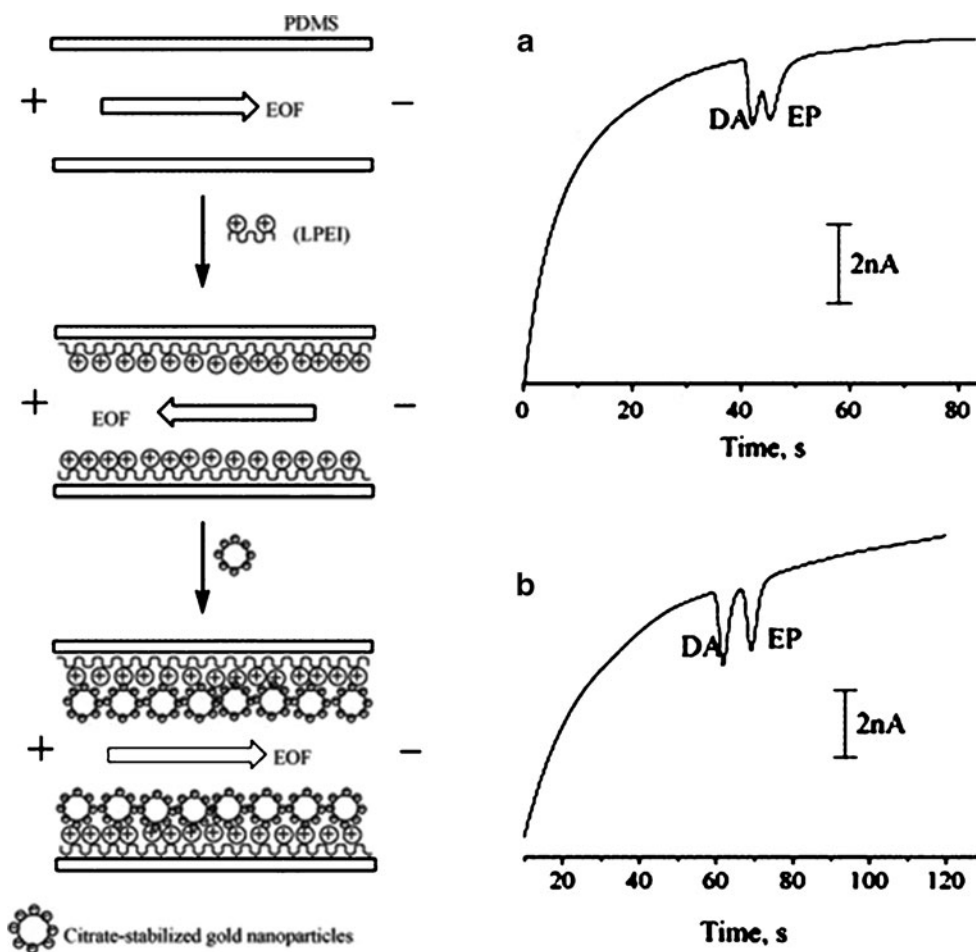
by using the LBL assembly technique (microchip structure: PDMS-PDDAC-Au NPs-cysteine) [104]. EOF of this channel can be reversibly switched by varying the pH of running buffer due to the amphoteric surface. The separation of DA and EP as well as arginine and histidine are performed by the proposed microchip.

Capillary electrophoresis

The use of alternating NPs was studied in CE systems [105–108] at least 10 years ago. However, the first article describing the real application of Au NPs for CE separation was reported in 2001 [109]. Grushka and co-workers used capillaries [109] and microchannels [110] coated with organically modified Au NPs to improve selectivity and control the EOF. Recently, Au NPs with either covalently functionalized carboxylic acid or amine surface groups were applied to CE separation of neurotransmitters (DA, EP, and pyrocatechol) [111]. Grushka and co-workers describe three Au NPs-specific mechanisms to explain the impact of the presence of Au NPs and their surface chemistry in separation.

CE is now a powerful analytical tool for biomolecule analysis, and some applications of Au NPs for the

separation and preconcentration of deoxyribonucleic acid (DNA) fragments and proteins are introduced in the following sections.

DNA fragments

Applications of polymer-adsorbed Au NPs for the separation of DNA fragments have been widely studied; particularly noteworthy is the contribution from Chang's group [112–118]. The analysis of double-stranded DNA (dsDNA) fragments by CE using poly(ethylene oxide) (PEO) solution containing Au NPs was reported for the first time by Huang et al. in 2003 [112], aiming at evaluating size dependence of the Au NPs and PEO on resolution and speed. Because the Au NPs enhance the interactions between DNA and PEO adsorbed on the NP surface, the high sieving ability of PEO is improved without markedly changing its viscosity. In other words, the separation efficiency obtained by using low viscosity PEO and Au NPs is comparable with that provided by more viscous PEO alone, but the analysis time is shorter in the less viscous matrix. Using different PEO solutions containing Au NPs ranging in diameter from 3.5 to 56 nm, Chang's group achieved reproducible, rapid, and

high-resolution DNA separations. The low viscosity PEO solution (<15 cP) offers a fast DNA separation power while providing comparable resolution to that of the high viscosity (2.0% PEO) solution. The authors suggested that the addition of 56 nm Au NPs to the long chains of 0.2% PEO polymer (8 MDa) was superior for the separation of dsDNA ranging in size from 8 to 2,176 bp within only 5 min (Fig. 8). In 2004, Chang's group presented the first example of the analysis of long dsDNA molecules by nanoparticle-filled capillary electrophoresis (NFCE) [113]. Au NPs are also modified with PEO through noncovalent bonding to form Au NPs/polymer composites (Au NPPs) of inorganic-organic hybrid nature. When compared with the free linear polymers, the polymers adsorbed on the Au NPs are stiffer and less extended, depending on the size of the Au NPs and the length of the polymer chain, and, thus, become slightly deformed under the flow. The separation of λ-DNA (0.12–23.1 kbp) fragments by NFCE was success-ful at pH 7.0. In addition, the ability to separate high molecular weight DNA markers (8.27–48.5 kbp) with plate numbers greater than 10^6 suggests that this novel method may hold great promise for the analysis of long stranded DNA molecules.

The feasibility of using the Au NPPs was further demonstrated by microchip CE performed by Chang's group; the chip material comprised poly(methyl methacry-late) (PMMA) with a 75-μm separation channel, and the chip is coated in sequence with poly(vinyl pyrrolidone) (PVP), PEO, and 13 nm Au NPs [114]. Chang's group also found that citrate stabilizes the Au NPs in 1.5% PEO as

Fig. 8 Separations of 10 μg/mL DNA markers V and VI using **a** 0.2% PEO (8 MDa) and **b** 0.2% PEO (8 MDa) containing 0.3×56 nm Au NPs. Electrophoresis conditions: PEO was prepared in 25 mM glycine, pH 9.0, containing 0.5 μg/mL EtBr; electrokinetic injection at 1 kV for 10 s; separation at 15 kV in a 40-cm-long (30 cm to the detector) fused-silica capillary with 365-μm O.D. and 75-μm I.D. (reprinted with permission from Ref. [112].)

well as affecting the electrophoretic mobility of DNA, and thus its concentration is an important parameter for controlling the analytical resolution and speed. However, one-layer coating of the PMMA with PVP was not effective in this case and the adsorption of DNA fragments led to poor repeat ability and resolution. Subsequently, the additional coating with three-layer of PEO and Au NPs allowed for much better results. To further investigate the separation mechanism, the migration of λ-DNA was monitored in real time by using a charge-coupled device imaging system when the capillary was filled with Au NPPs [115]. Chang et al. observed that the separation efficiency and speed for different DNA samples can be optimized further by using differently sized and shaped Au NPPs, or other NPs, and/or by adjusting the velocity of the hydrodynamic flow.

As mentioned above, the addition of suitable additives into low viscosity polymer solutions has provided a high-resolution, very efficient and simple method to improve dsDNA separation. However, the study on additives for single-standed DNA (ssDNA) sequencing is still lacking. In 2007, sieving matrices for ssDNA were first reported by Wang et al. based on Au NPs and a quasi-interpenetrating network composed of linear polyacrylamide and poly-N,N-dimethylacrylamide [119, 120]. Moreover, trace analysis of DNA by using preconcentration, microchip CE, and electrochemical detection in relation to Au NPs was reported by Shiddiky and Shim [121]. The aim of that study was to develop a simple, sensitive, and direct methods for analyzing DNA by integrating sample pre-concentration with a separation step on a microchip. Various experimental conditions, such as Au NPs concen-tration, water plug length, preconcentration time, detection potential, and separation field strength, that alter the analytical performances were investigated and optimized.

Proteins

The need for high resolution and more sensitive protein separation has become increasingly important due to the recent advances in molecular biology. Surfactant-capped Au NPs were utilized for the CE separation (e.g., NFCE) of acidic and basic proteins for the first time in 2006 [122]. This work introduced a simple approach for highly efficient separation using didodecyldimethylammonium bromide (DDAB) bilayer-protected Au NPs as dynamic coating additives. The bilayer structures of double-chained surfactants provide a greater surface coverage and better stability than single-chained surfactants. By using a DDAB-coated capillary, the separation of basic proteins was demonstrated by Lucy's group [123, 124]. The DDAB-coated capillary is more stable once the ionic strength of the background electrolyte is increased, resulting in improve-
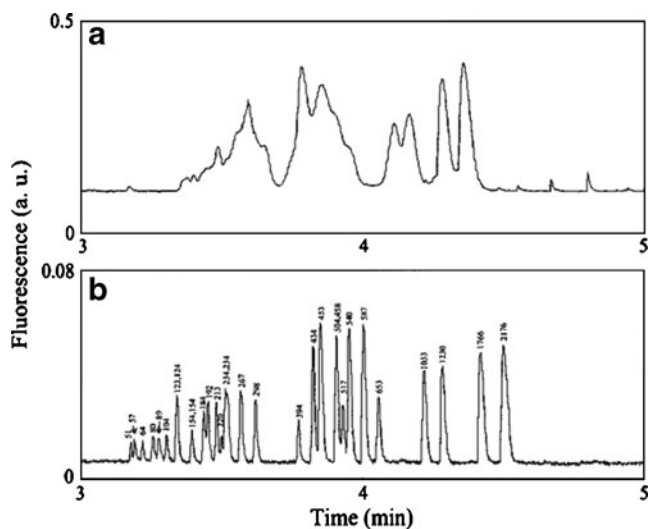
ment of peak efficiency and reproducibility of migration time. However, the adsorption of low isoelectric point (p*I*) proteins on the capillary wall is unavoidable because of strong electrostatic interactions with the positively charged DDAB head group. Therefore, poor separation efficiency and tailing of acidic proteins are only observed with DDAB-modified capillaries. In the effort to overcome this deficiency, DDAB-capped Au NPs are used as the pseudostationary phase for protein separation in NFCE. The authors suppose that the high affinity between proteins and modified Au NPs exists due to hydrophobic interaction and covalent conjugation of cysteine and lysine residues of protein onto Au surface. Successful separation of both acidic and basic proteins is performed under optimized conditions (at pH 3.5 with 10 mM phosphate buffer) with DDAB-capped Au NPs (Fig. 9). Moreover, the separation efficiency is then further increased by modification of the DDAB-capped Au NPs with neutral PEO molecules. Separation efficiency for proteins with p*I* ranging from 4.7 to 11.0 is achieved in the presence of 0.05% PEO, and demonstrates the advantages of high efficiency, excellent reproducibility, and stable EOF. Finally, several biological samples (i.e., saliva, red blood cells, and plasma) were analyzed by the proposed method. The authors concluded that separation performed by NFCE could be further optimized by using Au NPs with different size, shape, and concentration for proteomics applications and clinical diagnosis. In the subsequent study [125], they describe a method for enrichment and separation of acidic and basic proteins using the centrifugal ultrafiltration followed by NFCE. In comparison with PEO, cetyltrimethylammonium bromide, and polyvinyl alcohol, the addition of Au NPs in the separation buffer containing 1.6% PDDAC exhibits better stacking efficiency, greater separation efficiency, and shorter separation time. A several hundred-fold increase in sensitivity was observed through the combination of CE stacking and centrifugal ultrafiltration. The authors proposed a successful separation method even for egg white proteins, which have large differences in molecular weight and p*I*. This system should be used to concentrate a variety of compounds, such as catecholamines, indolamines, and peptides.

For the trace analysis of some biomolecules (i.e., peptides or proteins), the Au NPs-assisted sample preparation approaches have been developed and used to maximize the information content available by mass spectrometry (MS) [126, 127]. By modifying the Au NPs with cationic or anionic functional groups, Vanderpuije et al. [126] report that functionalized Au NPs systems behave as selective and efficient extraction agents for negatively or positively charged peptides, respectively. The improved efficiencies are mainly dependent upon peptide p*I* and solution pH. In order to reduce sample handling and minimize any
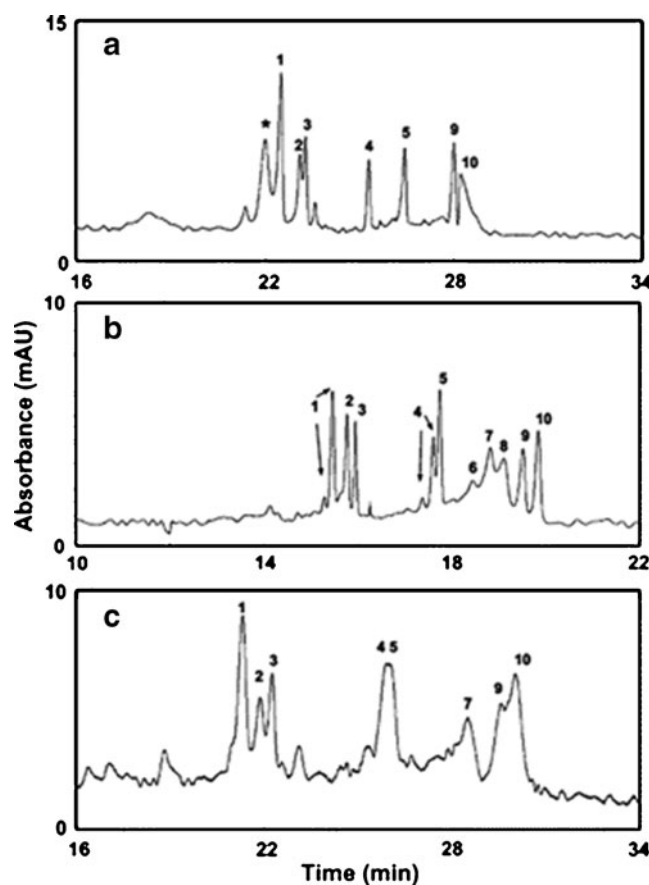

Fig. 9 Comparison of the separations of acidic and basic proteins under three different conditions at pH 3.5 using **a** DDAB-modified and **b**, **c** Au NP-modified capillaries. The separation buffer was **a** 25 mM phosphate buffer containing 0.1 mM DDAB, **b** 10 mM phosphate buffer containing 0.1 × Au NPs, and **c** 25 mM phosphate buffer containing 0.1 × Au NPs. Electrophoresis conditions: 80-cm-long capillary (60 cm to detector); applied voltage −8 kV; hydrodynamic injection at 20-cm height for 10 s; and direct UV detection at 220 nm. Peak identities: *1* α-chymotrypsinogen (5 μM); *2* ribonuclease A (5 μM); *3* trypsinogen (5 μM); *4* cytochrome c (5 μM); *5* lysozyme (2.5 μM); *6* BSA (0.5 μM); *7* carbonic anhydrase (1 μM); *8* ovalbumin (10 μM); *9* myoglobin (5 μM); *10* α-lactalbumin (5 μM) (reprinted with permission from Ref. [122])

associated sample losses, the Au NPs/peptide composites can be analyzed by matrix-assisted laser desorption/ionization (MALDI)-MS after extraction steps. The authors conclude that this feature could make these Au NPs suitable materials for targeted protein extraction from biological samples. However, Au NPs are also utilized in proteomics [127, 128]. For the rapid mapping of target protein, Chen et al. report an approach of using carbohydrate-encapsulated Au NPs as an affinity probe for the efficient protein identification by MALDI–time-of-flight (TOF) MS [127]. Target proteins could be affinity captured from a mixture by the nanoprobe-based affinity mass spectrometry (NBAMS) at the femtomolar level. The rapid NBAMS approach shows promise in profiling the proteome in a specific

ligand-fishing manner. Thus, Au NPs-assisted protein enrichment can be considered as a feasible alternative step in sample preparation to couple with various subsequent protein treatments. There are tremendous opportunities to be explored for nanobiotechnology applications.

Conclusions

This review outlines the potential of functionalized Au NPs and the many advances they have allowed in the separation and preconcentration of a variety of analytes. The modification of the Au surface with appropriate chemical species can improve the separation and preconcentration efficiency, analytical selectivity, and method reliability. This review systematically highlights the use of Au NPs as inorganic or organic-inorganic hybrid materials for the purpose of characterizations and applications. The demands of chemical analysis in modern biology, chemistry, medicine, environmental science, and industry require very high sample throughput and parallel analytical strategies. Optimization of electrophoretic and chromatography systems is part of this 'nanorevolution' but new challenges will still be faced in this field. While the increasing role of NPs in separation science is evident, the future will require greater control over the nanoparticle size, composition, self-assembly and, most importantly, integration of Au NPs-based hybrid material for innovative analysis.

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