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Review

Application of gold nanoparticles in separation sciences

The review article is devoted mainly to the description of applications of gold nanoparticles (GNPs) in separation sciences, especially in electromigration and chromatographic techniques. The applications of GNPs in particular separation methods, CE, microchip CE, MEKC, CEC, HPLC and GC, are classified according to the molecular size of the analytes from low-molecular-mass compounds *via* medium sized substances to biopolymers (proteins and nucleic acids). A very recent and promising utilization of GNPs for sample preparation, preconcentration and preseparation of selected analytes from complex matrices is presented as well. Moreover, in two introductory sections, typical preparation procedures of the GNPs and their modifications are presented and physicochemical and analytical methods employed for characterization of the native and modified GNPs are briefly introduced.

1 Introduction

Nanoparticles (NPs), *i.e.* particles with the dimensions in the range of units to hundreds of nanometers, recently attract an extensive attention in various fields of chemistry, physics, material science, medicine, and photonics, due to their unique physical and chemical properties [1–6]. NPs usually refer to a kind of material with a spherical-like appearance with a large surface-to-volume ratio and other fascinating properties derived from the "quantum size effect" [1]. The potential of nanostructured materials in separation sciences has gradually been recognized in recent

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Abbreviations: APTMS, 3-aminopropyl-trimethoxysilane; **DDAB,** didodecyldimethylammonium bromide; **FS,** fused silica; **FTIR,** Fourier-transform infrared spectrometry; **GMP,** gold microparticle; **GNP,** gold nanoparticle; **IPN,** interpenetrating network; **LBL,** layer-by-layer; **LPA,** linear polyacrylamide; **LPEI,** linear polyethyleneimine; **µGC,** microfabricated GC; **MPTMS,** 3-mercaptopropyl-trimethoxysilane; **NP,** nanoparticle; **ODA,** octadecylamine; **ODS,** octadecylsilane; **OT-CEC,** open-tubular CEC; **PAH,** polyaromatic hydrocarbon; **PDADMAC,** poly(diallyldimethylammonium) chloride; **PEO,** polyethylene oxide; **PMMA,** poly(methyl methacrylate); **PSP,** polystyrene particle; **SAM,** self-assembled monolayers; **SPR,** surface-plasmon resonance; **TEM,** transmission electron microscopy; **TGA,** thermal gravimetric analysis; **XRF,** X-ray fluorescence

years and significant advances have been achieved in CE, CEC, microchip CE, HPLC and GC separations of both low- and high-molecular-mass substances employing NPs as components of the separation media. The NPs serve either as permanent or as dynamic capillary inner surface coatings in CE, as stationary phases in CEC, as pseudostationary phases in partial filling or continuous filling mode in MEKC and as modifiers of stationary phases in LC and GC. The recent advances in application of NPs in separation sciences have been summarized in several reviews [7–13]. Several different types of NPs and nanostructures have been used successfully for the separation purposes, specifically, fullerenes, carbon nanotubes, polymers, silica, zeolite, lipid, latex, magnetic and non-magnetic metal oxides, metal-sulfides semiconductor, silver and gold nanoparticles (GNPs) [7–10, 13, 14]. This review article is solely focused on GNPs with the emphasis imposed upon their application in the separation arena. In addition, a concise description of typical preparation procedures of the GNPs and their modifications is presented and physico-chemical and analytical methods employed for characterization of the native and modified GNPs are briefly described.

2 Preparation of GNPs

2.1 Preparation and modification of GNPs in aqueous media

Stable colloidal solutions of GNPs are generally prepared in the presence of a stabilizer preventing aggregation and consequent precipitation of the GNPs. Stabilization based either on electrostatic or steric repulsion can be put into effect. The former mechanism utilizes anions delivering to

each NP a high negative charge; the latter uses various polymers to shield the NP surface. Majority of synthetic protocols for the GNPs relies on a chemical reduction of gold(III), specifically in the H[AuCl$_4$] or [AuCl$_4$]$^-$ form. A suitable reducing agent is added into a reaction mixture, most often citrate [15] or borohydride [16] or generated *in situ*, *e.g.*, by radiolysis of appropriate oligomers [17, 18]. In all the mentioned cases, an excess of reducing agents or their oxidation products serve as the GNPs stabilizer. Generally, two following distinct synthetic protocols according to the environment, in which the GNPs are prepared, can be distinguished:

Citrate reduction of gold(III) in water represents undoubtedly the most popular approach of the GNPs preparation [19–21]. In this method, introduced by Turkevich *et al.* in 1951 [15] and revised later [22], citrate serves as both a reducing agent and an anionic stabilizer. The method produces almost spherical particles over a tunable range of sizes covering 5–150 nm by varying stabilizer/gold(III) ratio. Other less frequent reducing agents including carboxylic acids (ascorbic, tannic, *etc.*), alkaline borohydrides, hydrazine, and hydoxylamine are used in the same manner as citrate [1].

The native (*i.e.* anion stabilized) GNPs prepared in aqueous solutions and electrostatically stabilized can be subsequently modified by a wide variety of ligands from thiols to phosphine derivatives and amines [1] by the incubation of NPs with the solution of an appropriate ligand. The goals of the modification are various but most frequently it is stabilization of the GNPs in a solution, adjustment of the surface properties of the NPs and immobilization of a specific selector. Thus, the GNPs with both positive and negative surface charge can be generated and the overall charge can be finely tuned by pH of the solution according to acidity constants (pK_a) if weak protolytes, *e.g.* carboxylates or amines, are anchored on the NP surface. For example, displacement of citrate and chloride by short and negatively charged thiol derivatives (3-mercaptopropionic acid, thioctic acid, *etc.*) can be followed by fuctionalization of NPs using negatively charged (carboxylate), neutral (crown and cyclodextrin), or positively charged (pyridinium and ammonium) ligands [23, 24]. Poorly water-soluble thiols (*e.g.* glutathione, thiopronine) were immobilized on GNP surface from methanol or methanol/acetic acid environment [25, 26]. Modification and stabilization of the GNPs can be accomplished not only by small molecules but also by polymers. Gittins and Caruso prepared GNPs coated with uniform polyelectrolyte multilayers by consecutively exposing the NPs to polyelectrolyte solutions of opposite charge [27]. Resulting polymer coating was so stable that dissolution of the gold core led to the hollow polymer particles.

2.2 Preparation and modification of GNPs in non-aqueous media

Gold(III) salt is transferred to toluene or chloroform [28] with phase-transfer agent (tetraoctylammonium bromide)

and reduced by borohydride in the presence of thiols, which strongly bind gold. This is the crucial point of the so-called Brust–Schiffrin method published in 1994 that allows the facile synthesis of thermally stable and air-stable GNPs ranging in diameter between 1.5 and 5.2 nm [29, 30]. Indeed, these modified GNPs can be repeatedly isolated and re-dissolved in common organic solvents without irreversible aggregation or decomposition. The ratio thiol/gold(III) used in synthesis controls the size of the GNPs. Further chemical modification is generally based on thiol-for-thiol ligand exchange [31] similarly as above described for aqueous environment. Modification with highly polar ligands, *e.g.* mercapto-PEG, can yield water-soluble GNPs [32].

3 Physico-chemical characterization of GNPs

3.1 General

In any application of GNPs, it is important first to determine their basic physico-chemical characteristics, such as *e.g.* size, shape, mono- or polydispersity, UV–vis and other spectral properties, electrokinetic potential and some other special parameters [33]. In this section, several physico-chemical and analytical methods employed for characterization of GNPs are briefly presented.

3.2 Transmission electron microscopy

Transmission electron microscopy (TEM) is a powerful and straightforward method for the determination of size (in several studies including size distribution) and shape of NPs [34]. It has been used for characterization of GNPs in most of the below cited applications of GNPs in separation sciences [35–60]. The advantage of this method stems from relatively direct observation of the real morphological shape and size of NPs and the capability of detailed analysis of digitized photographs utilizing software for image analysis. The most significant disadvantage of TEM is the impossibility to perform the measurement in liquid medium (aqueous, organic); thus, it is not an *in situ* analysis and the preparation of NPs for TEM imaging can affect formation of clusters of particles, aggregates, *etc.* The other disadvantage is related to the 2-D images obtained. Thus, the three dimensional description of the particle shape is only a reconstruction from 2-D images based on observation of a set of particles presuming their random orientation.

3.3 SEM

SEM technique is also frequently used [28, 37, 38, 43, 44, 46, 47, 53, 56, 61–64] for confirmation of adsorption/deposition and modification of GNPs on capillary/column walls and

several other morphological changes of capillary walls. The capillary has to be cut longitudinally prior to SEM investigation (the use of a razor blade for this purpose has been reported [37]).

3.4 Other microscopic techniques

Dark-field microscopy based on the intense Rayleigh scattering of submicrometer particles can be employed to characterize the adsorption of NPs on solid surfaces; *e.g.* silver-enhanced GNPs were detected on capillary wall using this technique [49]. The atomic force microscopy is another established method of nanostructural characterization but its application for characterization of NPs used for separation purposes is rather limited [53]. The above-mentioned TEM and SEM techniques are obviously preferred. For a survey of the past, presence and future of these and some other atomic-resolution spectroscopic imaging techniques, see the recent review [65].

3.5 UV–vis spectrophotometry

The other very common method is UV–vis absorption spectrophotometry [35–37, 39–42, 45–49, 52–55, 58–62, 66–68]. This spectral technique allows an *in situ* direct analysis of colloidal solutions (both hydrosols and organosols). The position of observed band maxima (typically in the range 500–540 nm) is related usually to particle size and in some cases both TEM data and surface-plasmon resonance (SPR) maxima are given. However, the position of SPR maximum cannot be directly related with the particle size of the NPs and other factors have to be considered [69]. The interpretation of UV–vis spectral data should be based on a physical model to relate the measured spectral curves to physico-chemical properties of GNPs. A thorough study of optical properties of gold hydrosols was carried out by Turkevich *et al.* already in 1954 discussing the effects of particle size, particle shape, aggregation, chemical composition and adsorbed ions [35]. A comparison of experimental spectral data with Mie's theory was presented; particle sizes ranged from 7.5 to 160 nm; shape parameters were derived from electron microscope. Negligible effect of particle size in the range of small particles (7.5–33 nm) on SPR maximum (522 nm) was described. Later experimental and theoretical studies of size and temperature dependence of plasmon absorption of colloidal GNPs (size range 9–99 nm) have shown very subtle effect of particle diameter on SPR maximum in the similar range (SPR maximum at 517, 520 and 521 nm corresponds to diameters 8.9, 14.8 and 21.7 nm, respectively) [36]. Under routine experimental conditions used in UV–vis spectrophotometry (*e.g.* slit-width, scan rate, *etc.*), a shift of 1 nm cannot be reliably and repeatedly resolved; it means that for particles smaller than 20 nm an accurate value of particle diameter is inaccessible under routine characterization measurement. The bandwidth of

SPR band depends on particle size, but in a complex way; it decreases with increasing particle size for small particles (smaller than 22 nm) and then it increases. Thus, the bandwidth is influenced both by the particle size itself and by polydispersity of GNPs system. The shifts of SPR maxima and shape changes of SPR curves can be caused by change in particle size/shape, formation of aggregates and/or adsorption of modifying molecules. It is difficult to distinguish these effects and any quantitative relation does not exist at present. For example, an addition of protein molecule to 13 nm GNPs (SPR maximum at 520 nm) causes a shift of only ca 7 nm (to 527 nm), while the 100 nm aggregates were shown from TEM images. Hence, from the point of view of morphological GNPs characterization, the SPR data should be supported with TEM analysis.

3.6 Fourier-transform infrared spectrometry

Fourier-transform infrared spectrometry (FTIR) allows to study the molecular species deposited on GNPs. Both the classical transmittance technique (KBr pellets [38], GNPs dropped on NaCl window [47]) and modern reflection technique (either attenuated total reflectance (ATR) [50, 62, 64, 70] or diffuse reflectance (DR) [63]) are applied. For example, purity of dodecanethiol monolayer-protected GNPs was tested by using TLC and FTIR collecting transmission spectra of the prepared KBr pellets [38]. In the case of octadecylamine-capped GNPs (ODA-GNPs), the attenuated total reflectance technique was selected to acquire FTIR spectra verifying the presence of ODA on ODA-GNPs modified inner surface of the capillary column [62]. The diffuse reflectance method was used to measure the IR spectrum of self-assembled monolayers (SAM) on the gold-coated polystyrene particles (PSPs), in which the unmodified gold-particles were employed as [63]. Of course, the pure GNPs do not exhibit specific FTIR spectral features of gold itself but the bands corresponding to the surface stabilizing ions (or other species) can be observed.

3.7 X-ray fluorescence

To verify the adsorption of GNPs on a capillary wall a combination of SEM with energy dispersive X-ray fluorescence (XRF) was used [37]. The authors claimed that "characteristic gold peak (1.74 keV) in addition to the expected silicon peak (2.12 keV) was observed". In fact, the 1.740 keV is a $K_{\alpha 1}$ line of silicon, 2.123 keV is an $M_{\alpha 1}$ peak of gold (X-LabPro v. 4.5, Spectro Germany). Energy dispersive XRF demonstrate that GNPs were grown successfully onto the capillary column through the self-assembly strategy, exhibiting peaks at 9.6 and 11.3 keV [61]; the positions are a little bit shifted compared with reference data of gold (9.713 and 11.440 keV, X-LabPro v. 4.5, Spectro).

3.8 X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy was applied to confirm the presence of GNPs on chitosan–GNPs–albumin-coated poly(dimethylsiloxane) (PDMS) surface [50]. The peaks at 89.5 and 93.5 eV were ascribed to the characteristic peaks of GNPs.

3.9 Molecular fluorescence

Several studies used steady-state fluorescence spectra to monitor the fluorophores present in modified GNPs systems [41, 51, 66, 67]. Changes in molecular fluorescence of modifying agent can be used to estimate the excess of fluorescent molecules in the systems with GNPs [41]. Fluorescence spectra of supernatants after various centrifugation times of GNPs systems and for various particle sizes were studied in detail [66].

3.10 Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) enables to study the thermal stability of modifying/deposited layers and to calculate the mass ratio of modifying substances and GNPs core [38, 43, 56]. The mass losses occur in a broad temperature range from 90 to 450°C. Thus, the temperature limits of modified GNPs are determined demonstrating limited capabilities to use such systems for GC. For example, the maximum operating temperature for dodecanethiol-GNPs was estimated at about 150°C [43].

3.11 Other analytical methods

An Ubbelohde viscometer was used to monitor viscosities in the absence and presence of GNPs [54, 55]. Intrinsic viscosities in dilute solutions would augment a little with the contents of GNPs. The contents of the GNPs in composites were measured by using atomic absorption spectrometry [54, 55]. The interactions of GNPs with polymers were confirmed using differential scanning calorimetry [54, 55]. X-ray powder diffraction analysis revealed that these micrometer gold spheres are produced by aggregation of nano-sized gold precursors [64]. The specific surface area of gold particles was calculated using sorption data; N_2 sorption isotherms were obtained at −196°C. The carbon content in modified systems was determined using elemental analysis. Liu *et al.* employed MS to characterize the surface coverage of carbon on the GNPs films [61]. Thermal desorption atmospheric-pressure-ionization technique was applied. Important characteristics, such as *e.g.* charge, size, electrokinetic potential and electrophoretic mobilities of the charged NPs can be obtained by electromigration methods [60, 71, 72], for more details see the recent review [73].

To summarize this section, TEM is very useful and often applied method to characterize GNPs size and shape. SEM technique is applicable to describe the formation of GNPs layers on capillary walls. UV–vis spectrophotometry is easy-to-measure method but the sound interpretation of data is rather difficult and multiple effects should be considered. The other analytical techniques (*e.g.* XRF, X-ray photoelectron spectroscopy, atomic absorption spectrometry, FTIR, MS, TGA) are used rarely, although their real potential to characterize complex systems embracing GNPs might be revealed in the future.
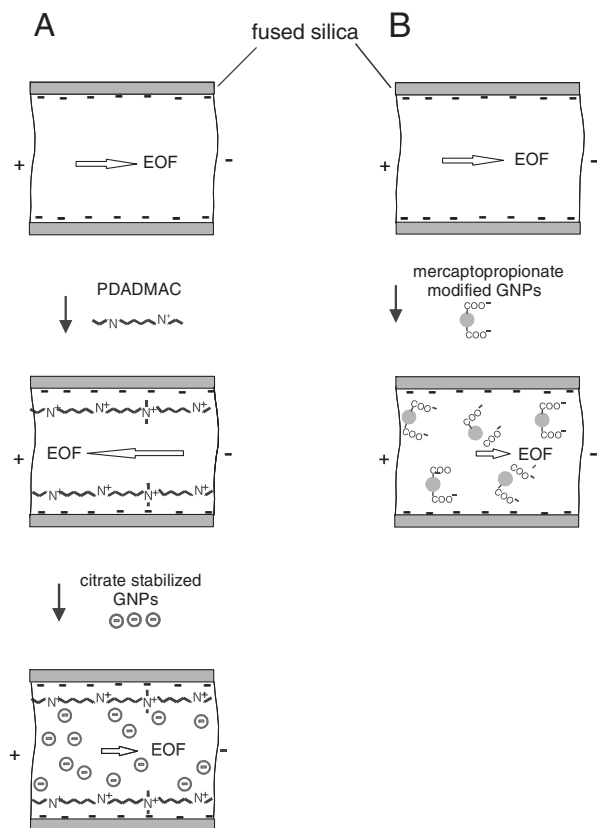
4 Applications of GNPs in separation sciences

4.1 CE, EKC, and microchip CE

4.1.1 Analysis and separation of low-molecular-mass compounds

The first article describing the application of stabilized GNPs for CE separation was published only in 2001 [37]. The authors prepared aqueous gold colloidal dispersions by frequently used citrate method relying on reduction of $AuCl_4^-$ by citrate ions. Two different GNPs species were used as additives to the BGE to generate a pseudostationary phase. First, unmodified citrate stabilized GNPs (∼18 nm) in conjunction with capillaries treated with poly(diallyldimethylammonium chloride) (PDADMAC) were prepared, see Scheme 1A. Acetate buffer adjusted to pH 5.0 served as the BGE. Second, sodium 3-mercaptopropionate GNPs (∼5 nm) were synthesized and used in combination with untreated fused silica (FS) capillaries. In the latter case, phosphate buffer (pH 6.4) was used as the BGE. The structural isomers of aromatic acids and bases were employed as the model analytes to study the effect of GNPs on the separation.

Modification of the inner capillary wall with the homogenous positively charged PDADMAC layer covered the silanol groups and reversed cathodic EOF to stabilized anodic EOF providing more repeatable migration times for toluidines, aminophenols, and toluic acids even without the GNPs in the BGE. Moreover, PDADMAC-modified capillaries yielded more efficient separations with more symmetrical peaks. Passing the citrate-stabilized GNPs through the PDADMAC-modified capillary resulted in the electrostatic adsorption of the negatively charged GNPs on the positively charged walls. Since the adsorbed layer of the citrate-stabilized GNPs was negatively charged, the EOF was reversed again back toward the cathode. It was shown experimentally that low concentration of the GNPs in the BGE was sufficient to occupy the great majority of the available adsorption sites on the PDADMAC layer. The presence of GNPs in the BGE changed both the EOF mobility of the BGE and apparent (observed) mobility of the analytes. The largest

Scheme 1.

changes in the separation selectivity occurred at low concentrations (∼0.01–0.02 nmol/L) of GNPs in the BGE (with respect to the concentration range studied, 0–0.4 nmol/L). Discussing citrate-stabilized GNPs, it is important to mention that the authors' attempt to use citrate-stabilized GNPs in the BGE without PDADMAC in the capillary was unsuccessful because of low GNPs stability in the BGE.

On the other hand, solutions of 3-mercaptopropionate stabilized GNPs were quite robust and they did not require combination with PDADMAC-coated capillaries, see Scheme 1B. Since the negatively charged analytes gave asymmetric and broad peaks in that system, the authors studied only the behavior of cationic analytes. Similarly to PDADMAC-citrate stabilized system, the initially introduced GNPs occupied the great majority of the available sites on the capillary wall and any further increase of the GNPs concentration in the BGE did not provide new interaction sites for the analytes. The greatest change in selectivity occurred again at low GNPs concentration (∼4 nmol/L) within the tested concentration range (0–60 nmol/L). The presence of the thiol-stabilized GNPs in the BGE improved the precision of the migration times and separation efficiency. The authors ascertained that although both the citrate and the thiol-stabilized GNPs gave more efficient and more repeatable CE separations, the thiol-stabilized GNPs resulted in better CE systems.

Further, still in 2001, a successful separation of aminophenols was achieved on a PDADMAC-GNPs-modified glass chip [74]. The resolution and the plate count in the treated channel were double the values obtained with the untreated channel. The electrochemical detection and the quantitation of the analytes were not affected by the GNPs. The lifetime of the PDADMAC-GNPs-coated chip stored in water was longer than one month and the day-to-day repeatability of migration times was also good (RSD < 3%). Recently, the GNPs covalently functionalized by carboxylic acid or amine surface groups have been applied to CE separation of neurotransmitters (dopamine, epinephrine, and pyrocatechol) [60]. Amino-derivatized GNPs increased the mobility of the neurotransmitters in 10 mM tetraborate BGE whereas a smaller opposite effect was observed for carboxyl-modified GNPs.

The application potential of NPs, including GNPs, for CE analysis of both low-molecular mass compounds as well as of the below high-molecular-mass substances has been critically evaluated by Zhang *et al.* [75]. They concluded that the NPs suitable for use in CE should meet the following requirements: (i) acceptable stability in variable BGEs, (ii) discriminating interactions with the analytes, (iii) different mobility than that of EOF, (iv) equal mobility to prevent band broadening, (v) low mass-transfer resistance, (vi) no disturbance on detection, and (vii) large surface area.
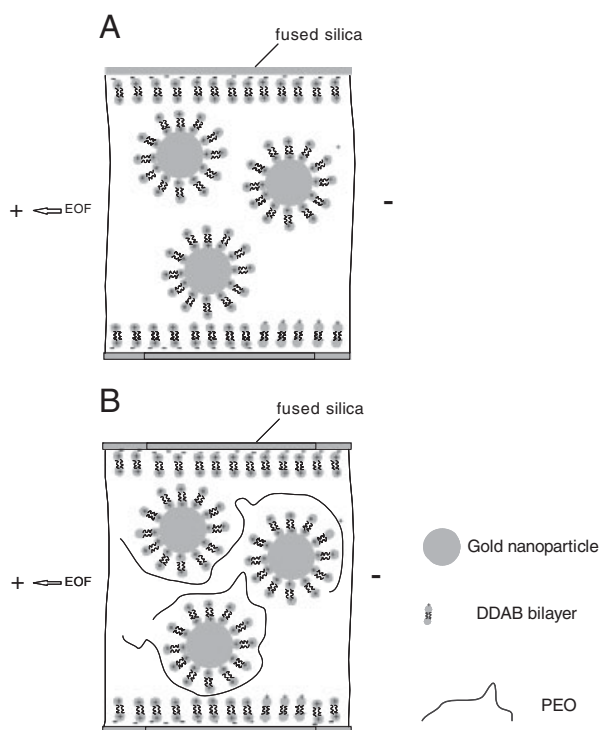
4.1.2 Analysis and separation of high-molecular-mass compounds

4.1.2.1 Proteins

Modified GNPs were utilized for the CE separation of acidic and basic proteins for the first time only in 2006 [49]. The work resulted from the positive experience of other authors with the bilayer structures of double-chained surfactants in CE separation of proteins. At first, the inner FS capillary wall was modified by a bilayer of the didodecyldimethylammonium bromide (DDAB). This coating provided a greater surface coverage and better stability than single-chained surfactants. The stability of DDAB-coated capillary increased with increasing ionic strength of the BGE, resulting in improved separation efficiency and repeatability of migration times. However, proteins of low pI tended to adsorb on the capillary wall because of strong electrostatic interactions with the positively charged DDAB head group. Thus, acidic proteins provided poor separation efficiency and tailing. In the effort to overcome this deficiency, DDAB-capped GNPs (6 nm) were added to the BGE as the pseudostationary phase, see Scheme 2A. High affinity of proteins to the modified GNPs was expected due to hydrophobic interactions and covalent conjugation of cysteine and lysine residues of proteins to gold surface. Successful separation even of the acidic proteins was achieved at pH 3.5 with 10 mmol/L phosphate buffer containing DDAB-capped GNPs. Moreover, the modified-GNP-coated capillary gener-

ated more stable and faster reversed EOF than the capillary modified solely with DDAB. The separation efficiency remained unchanged for tens of consecutive injections for a 5-day period without regeneration of the capillary. The separation efficiency was then further increased by modification of the DDAB-capped GNPs with neutral high-molecular-mass poly(ethyleneoxide) (PEO), see Scheme 2B. The resulting GNPs composite provided a good capability to interact with the proteins in analyzed samples. Separation efficiency for the basic proteins ranged between 6×10^5 and 10^6 plate/m and a good separation of five tested acidic proteins was achieved in the presence of PEO. Finally, several biological samples were analyzed by this new method (saliva, red blood cells, and plasma) with moderate outcome.

In the subsequent article devoted to the GNPs-assisted CE separation of proteins [58], the FS capillary wall was coated with PDADMAC and the BGE contained also PDADMAC plus one of the following additives, PDAD-MAC-modified GNPs, PEO, CTAB and poly(vinyl alcohol). The increasing concentration of PEO in the BGE led to significant decrease of the migration times of acidic proteins. PEO effectively suppressed adsorption of acidic proteins on the PDDA-coated capillary. However, the separation efficiency also decreased with increasing PEO concentration. Poly(vinyl alcohol) and CTAB provided unsatisfactory results in terms of separation speed, selectivity, repeatability and efficiency. On the contrary, PDDA-modified GNPs added into the BGE significantly enhanced separation efficiency and speed of analysis. The results
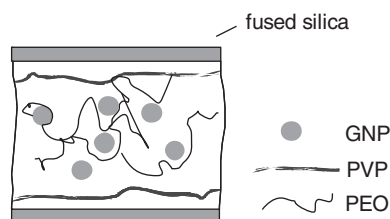
implied that PDDA-modified GNPs showed a stronger interaction with acidic proteins than PDDA, thus, the proteins adsorbed on the PDDA-coated capillary wall were released faster in the presence of the modified GNPs. Moreover, sample stacking was obtained and a large-volume injection (110 nL) was feasible conserving the separation efficiency in the range 10^4–10^5 plates/m.

4.1.2.2 DNA fragments

Applications of GNPs for the separation of DNA fragments are relatively numerous, particularly due to the high activity of Chang's group in this area [39–42, 45, 48]. For CE separations of DNA, the solutions of entangled and non-cross-linked polymers are often used as they possess lower viscosity and better flexibility than the cross-linked gels, which allows for automatic filling and replacement of sieving matrices [76]. As the behavior of the polymer is essentially determined by its chemical structure and morphology, copolymers and mixtures of homopolymers of different sizes have been applied for the DNA separations [77]. Searching for low-viscosity polymer solutions with high sieving ability still remains an important issue for high-throughput DNA analysis [78]. One approach potentially leading to the fulfillment of the mentioned goal relies on the mixing of suitable additives into low-viscosity sieving matrices.

The effect of the GNPs added to the BGE containing PEO on the CE separation of DNA was studied for the first time by Huang *et al.* in 2003 [39]. It is well known for a long time that some polymers, similarly as capping agents such as citrate, are able to prevent aggregation and agglomeration of GNPs in solution. This was verified for PEO, often used for CE separations of DNA. Further, it was found that FS capillaries had to be dynamically coated with PVP prior to their use for CE. PVP suppresses EOF, minimizes interactions between the capillary wall and the GNPs, and stabilizes the GNPs. The influence of the PEO chain lengths (2, 4 and 8 MDa) and the GNPs size (3.5–56 nm) on the separation was studied. The schematic representation of the system is shown in Scheme 3.

Very good and fast (5 min) CE separation was obtained for small DNA fragments (upto ∼2 kbp) after the addition of GNPs to the sieving matrix. The positive influence of the GNPs on separation efficiency is very cogent. The authors concluded that the addition of large GNPs (56 nm) to the long chains of 0.2% PEO (8 MDa) was superior for the

Scheme 2.

Scheme 3.

separation of small size dsDNA. This low-viscosity PEO solution (<15 cP) offered a fast DNA separation while providing comparable resolution to that using highly viscose 2.0% PEO solution. The effect of the GNPs on the separation of large DNA fragments (up to ~10 kbp) was less pronounced but still significant.

The feasibility of the concept was further demonstrated by microchip CE using a poly(methyl methacrylate) (PMMA) 75 µm separation channel dynamically coated in sequence with PVP, PEO, and 13 nm GNPs [40]. Sole coating of the PMMA with PVP was not effective in this case and the adsorption of DNA fragments led to poor repeatability and resolution. However, the additional subsequent coating with PEO and GNPs allowed for much better results.

Comparing results presented in [39] and [40] it can be concluded that using PVP-PEO-GNPs-coated PMMA chip provided a comparable resolving power for DNA markers V and VI ranging from 8 to 2176 bp as the classical CE in 40-cm long FS capillary modified with PVP. However, 1.5% PEO(GNP) requiring a high pressure to fill and flush the microchannel had to be used in the case of the chip separation as the sieving matrix in contrast to 0.2% PEO(GNP) for CE. Moreover, since diffusion coefficient of DNA and dispersion are smaller in high-viscosity solution, it was concluded that the loss of efficiency on the chip was due to the undesirable interaction of DNA with the modified chip walls because of incomplete coating. All of this stimulated the research oriented on the improvement of the wall-coating procedure in PMMA based chips and on the development of sieving matrices of lower viscosity [48]. The experiments showed that indeed highly repeatable, high-resolving, and high-speed CE separations of DNA fragments were attainable. However, repeated wall coating with PEO and PVP twice, and then coating with PEO(GNPs) along with the application of the solution of 0.75% PEO containing 56 nm GNPs filled in the channel was necessary [48].

Previous CE studies [39, 40] indicated that the relative sizes of GNPs and PEO and BGE composition are important factors in DNA separation. In the following work, these and several other parameters were further studied in detail, specifically, the effect of pH, concentration of GNPs, PEO, and ethidium bromide on the separation was investigated [42]. Based on the gained knowledge, the separation of DNA ranging in size from 21 bp to 23.1 kbp was obtained in 7 min under the optimal separation conditions with excellent repeatability for migration times.

Successful CE separation of even longer fragments of dsDNA (upto 48.5 kbp) was obtained in GNPs-filled FS capillary in 2004 [41]. As the separation of DNA by CE using a GNPs-coated capillary filled with GNPs dissolved in glycine–citrate buffer was unsuccessful, an alternative approach was sought. This led to the preparation of GNP/polymer composites (GNP-PCs). To avoid aggregation of the GNPs and to allow strong interaction with the DNA molecules, the GNPs were modified with PEO through noncovalent bonding to form GNP-PCs. The neutral GNP-PCs slowed down the electromigration of DNA molecules, and in comparison with linear polymer solutions, the GNP-PCs provided better separation efficiency in shorter times for long dsDNA fragments. The method produced plate numbers greater than 10^6, and, in contrast to the used micro- and nanofabricated devices, it was much simpler and affordable. Separation mechanism was proposed and the authors concluded that the resolving power of the separation depends on interactions between DNA and PEO adsorbed on the GNPs.

Further, the hydrodynamic force was employed in parallel with the electric field force for the separation of long DNA molecules [45]. When compared with the results of the previous study [41], the new technique had advantages of better resolution, rapid separation, excellent repeatability, ease of preparation, and fewer undesired peaks from aggregation, cleavages, and spikes due to scattering. The migration of λ-DNA was monitored in real time using a CCD imaging system to investigate the separation mechanism. It was concluded that the separation efficiency might be potentially further improved by using differently sized and shaped GNPs, or other NPs, and/or adjusting the velocity of the hydrodynamic flow. According to the authors, the use of polymer-adsorbed GNPs was fully competitive with the methods employed conventionally for the separation of long DNA fragments.

The experience gathered by Chang's group with GNPs contained in PEO matrices in CE separations of dsDNA was fully utilized by Chen *et al.* in an important medical application, the genotyping of α-thalassemia deletions using multiplex PCR and GNP-filled CE [79]. Under optimized conditions, simultaneous diagnosis of five common α-thalassemia deletions was possible and the results showed a good agreement with those obtained by gel electrophoresis. However, in contrast to the time-consuming and labor-intensive gel electrophoresis, CE possesses a potential of a high-throughput method. From the methodical point of view, the article is interesting in the application of the commercial DB-17 capillary, coated with (50%-phenyl)-methylpolysiloxane, instead of PVP or PEO dynamically coated FS capillary used by Chang's group. Thus, the long preparation time needed for the PVP-coated capillary was avoided without any decrease in the separation performance. The described diagnostic CE method was found to be simple, fast and reproducible.

Trace analysis of dsDNA using preconcentration, microchip CE and electrochemical detection utilizing GNPs was developed by Shiddiky and Shim [53]. The hydroxypropyl cellulose matrix modified with GNPs was applied to improve both preconcentration and separation of fragments of dsDNA ladder (upto 100 bp). Field-amplified sample stacking and field-amplified sample injection methods were used for DNA preconcentration in microchannels for the first time. Moreover, the conducting polymer-modified electrode was also doped with GNPs to enhance detection. The conducting polymer/GNP layer
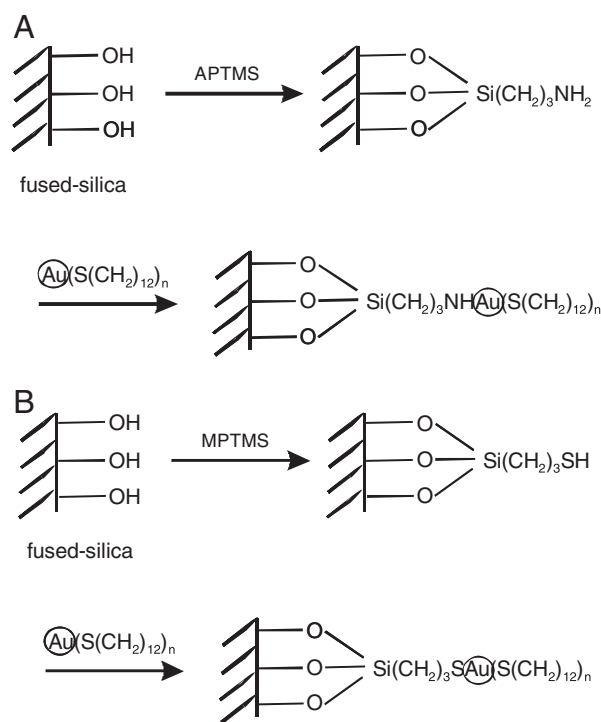
acted as electrocatalyst for the direct detection of DNA. This detection scheme eliminated the need for labeling of DNA and GNPs. The microchip consisted of two preconcentration channels to facilitate sample preconcentration by field-amplified sample stacking and modified-field-amplified sample injection steps and a separation channel for the microchip gel electrophoresis. Various experimental conditions affecting the analytical performances, such as buffer concentration, water plug length, hydroxypropyl cellulose concentration, GNP concentration, preconcentration time, electrode geometry, and separation field strength were addressed and optimized. The attomole sensitivity was obtained with a good perspective for further improvement. Diagnosis of diseases by detecting the trace level markers at the early stage of diseases was envisaged by the authors as relevant application of the method.

As mentioned above, the addition of suitable additives into low-viscosity polymer solutions has proved to be a very efficient and simple method to improve dsDNA separation. However, the studies on additives for ssDNA sequencing were deficient. Only in 2007, Wang's group studied in detail the influence of GNPs present in quasi-interpenetrating network (quasi-IPN) matrices on ssDNA sequencing performance [54, 55]. The non-cross-linked quasi-IPN matrix was formed by linear polyacrylamide (LPA) and poly-*N,N*-dimethylacrylamide (PDMA). LPA provided high sequencing ability and read length, however, it had no self-coating ability to reduce the EOF and adsorption of DNA on the capillary walls. On the other hand, PDMA had excellent self-coating ability but poor sieving performance. As no single known homopolymer completely met the requirements, the mixed matrix was designed and experimentally tested with promising results [80]. In the effort to improve the separation performance, about 40-nm GNPs were added into quasi-IPN to form polymer/metal composite matrices [55]. It was experimentally shown that GNPs helped to stabilize the sieving network. The authors proposed that GNPs interacting with polymer chains might act as physical cross-linking points. The sequencing results on ssDNA clearly showed the improved performance of quasi-IPN/GNPs composite as compared to the quasi-IPN without GNPs. In the next article, the authors studied the effects of GNPs size (20, 40, and 60 nm) and different viscosity-average molecular masses of LPA (1.5, 3.3, and 6.5 MDa) on ssDNA sequencing performance [54].

In summary, the presence of the GNPs in the separation media for CE analysis of DNA provided advantages of high resolution, separation efficiency and speed, very good repeatability, long shelf life, and easy automation. The latter aspect making the GNPs containing matrices particularly interesting for automated DNA separations in microfluidic systems, in which low-pressure loading of sieving matrices is highly desirable, is related to the fact that less viscous LPA matrix solutions containing GNPs proved comparable or better separation performance than highly viscous concentrated and high-molecular-mass LPA matrices without GNPs.

4.2 CEC

The first covalent immobilization of GNPs on the FS capillary wall and their application in open-tubular CEC (OT-CEC) was published by Glennon and coworkers in 2003 [28]. They synthesized dodecanethiol GNPs in the first step. Next, these particles possessing hydrophobic surface were covalently anchored on the prederivatized 3-aminopropyl-trimethoxysilane (APTMS) or 3-mercaptopropyl-trimethoxysilane (MPTMS) capillary, see Scheme 4. The electrochromatographic properties of the GNPs-modified capillaries were investigated using a RP test mixture of neutral compounds, thiourea (EOF marker), benzophenone, biphenyl, and pyrethroid pesticides. They also experimented with ''loosely coated'' columns prepared by passing of dodecanethiol GNPs through a bare FS capillary pretreated only with NaOH/HCl. The effect of pH and applied electric field strength on EOF was studied, as well as the influence of an organic modifier on the chromatographic retention. Plots of the logarithm of retention factor *k* of benzophenone and biphenyl *versus* the methanol content in the mobile phase exhibited linear relationships, confirming RP behavior. Efficient separation of the both analytes was obtained on the Au-MPTMS and the Au-APTMS capillary columns. Plate numbers for benzophenone and biphenyl increased significantly with capillary id decreasing from 50 to 30 μm. The run-to-run repeatability of the retention factors for the tested compounds on GNPs-covalently coated capillaries as well as their long-term stability were found acceptable. On the contrary, the GNPs loosely coated capillaries suffered

Scheme 4.

from rapid deterioration of separation capabilities due to washing-off of the GNPs from the column. This fact clearly demonstrated the necessity of a suitable coupling agent to prevent the GNPs leakage from the capillary wall.
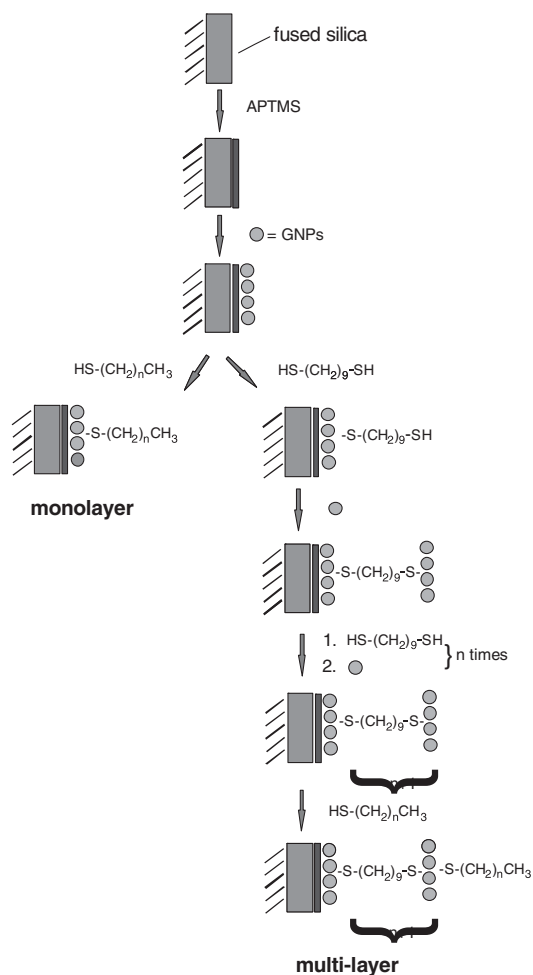
The OT-CEC concept based on the GNPs-coated capillaries has many positive aspects. However, one serious weak point of the GNPs-modified capillaries is related to their low loading capacity and relatively low stationary-to-mobile phase ratio since only the inner capillary wall is modified by GNPs. Being well aware of this limitation, the researchers of the Glenonn's group followed the approach of Pesek *et al.* [81–83] and applied their etching method as a means of significant increase of wall surface area (by a factor of up to 10^3) and, consequently, enhanced interaction between the analytes and the immobilized stationary phase [46]. FS capillary (50 µm id) was etched with ammonium hydrogen difluoride before its subsequent modification with MPTMS and covalent immobilization of dodecanethiol-GNPs (2–3 nm). The process of etching was easily followed by optical and SEM. The significantly increased surface area of the inner wall was found to be uniform and well defined. Interestingly, the etching procedure led to the decrease of EOF with respect to the bare FS capillary, which was explained by the reduced amount of free silanols on the surface after the etching process. The study of the influence of pH and methanol content in the mobile phase on the retention factors of the RP test mixture composed of thiourea, naphthalene and biphenyl, revealed that both etched and non-etched GNPs-coated capillaries displayed EOF profiles similar to that of bare FS capillary. Retention factors were increased greatly on the etched GNP capillary (3.2 × for naphthalene, 1.8 × for biphenyl). Both etched and non-etched GNPs-modified capillaries provided repeatable retention factors, they were stable for up to 3 months when not used and stored in water and there was no observable change in separation efficiency for at least 100 injections.

An alternative approach to the capillary etching rendering an increased phase ratio is the sol-gel technique [47], which provides large surface stationary phases with high stability, mass loadability and separation efficiency. Despite the preparation process usually includes five steps: hydrolysis, condensation and polycondensation of sol-gel precursors, casting of the gel, aging and drying; still it can be considered relatively simple. Here, the sol-gel solution was prepared by controlled hydrolysis of MPTMS. The coated capillary was subsequently modified once more with MPTMS and finally dodecanethiol-GNPs were applied. Testing analyte mixtures included besides the RP solution also several drug substances (propiophenone, benzoin and warfarin). The results obtained with sol-gel-based GNPs-modified capillaries were in many aspects very similar to the data measured in the chemically etched modified capillaries as mentioned above. For instance, the dependences of the EOF mobility *versus* methanol content in the mobile phase and EOF mobility *versus* pH of the mobile phase showed the same features for both types of capillaries. Similarly, the

run-to-run repeatability of the retention factors and column stability was found to be very good. All in all, it was demonstrated that both the etching and the sol-gel approach can provide capillaries with higher phase ratio and thus, allowing for better separation efficiency and selectivity of important analytes, such as polyaromatic hydrocarbons (PAHs) and drugs.
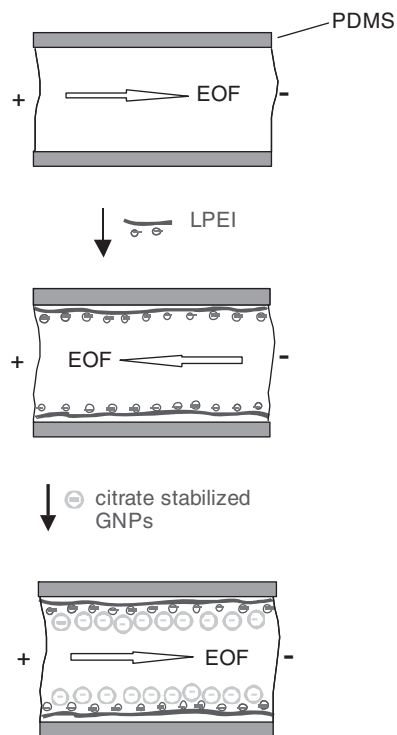
Three neutral steroid drugs, testosterone, progesterone and testosterone propionate, were successfully separated on OT-CEC columns prepared by alkanethiol self-assembly and dithiol layer-by-layer (LBL) self-assembly process embracing GNPs [61]. First, the FS capillary was modified with APTMS, and then GNPs (~17.8 nm) were introduced into the capillary. Next, the covalently immobilized GNPs were 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 GNP surface with 1,9-nonanedithiol and GNPs was employed providing a multilayered film on the inner capillary wall, see Scheme 5. The effect of GNPs layers on the CEC separation was studied and it was found that retention of the hydrophobic analytes significantly increased with the number of layers created in the capillary. As the separation efficiency started to decrease for layer numbers above four, the four-layer film was considered to be the optimal arrangement in subsequent experiments. The effect of the alkanethiol chain length on the behavior of the analytes agreed well with the expectation of the authors and they concluded that C18 chain self-assembled on the four-layer GNPs structures provided the highest retention and the best efficiency for separation of the steroids. The experiments oriented to the evaluation of the influence of the ACN content in the mobile phase on the separation strongly supported the authors' thesis that the retention of steroids is controlled primarily by RP mechanism. EOF velocity in the four-layer modified capillary was of the same direction but much lower than that in the bare FS capillary. Bonding of APTMS moieties to the silanol groups on the inner capillary surface is probably responsible for this reduction of EOF. The chemical stability of the synthesized stationary phases in ACN and aqueous solutions of various pH (3–11; pH adjusted with 1 mol/L HCl, NaOH, respectively) and also repeatability of the chromatographic retention was ascertained to be very good.

Successful separation of neurotransmitters dopamine and epinephrine on GNPs-modified PDMS microchip prepared by electrostatic LBL assembly technique was demonstrated by Wang *et al.* [51]. Amperometric detection with a single carbon fiber electrode was used. PDMS has many useful properties, however, its several defects in separation field are also well known, *e.g.* EOF instability and a strong tendency to adsorb analytes due to very high hydrophobicity. Suitable covalent or dynamic coating can deliver more favorable physico-chemical characteristics to the chip surface. The dynamic coating with polyelectrolytes was proposed and tested to influence EOF and shield
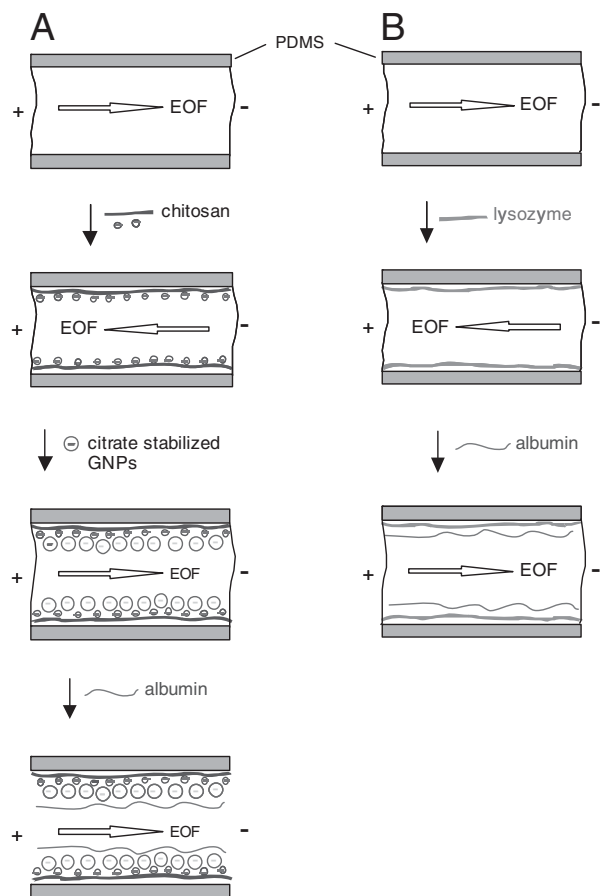
Scheme 5.

Scheme 6.

analytes from the microfluidic channel sorption. The authors investigated four polyelectrolytes, linear poly-ethylenimine (LPEI), PDADMAC, poly(allylamine) hydro-chloride and chitosan. Coating with a polyelectrolyte formed a cationic surface electrostatically adsorbed onto the channel walls resulting in a reverse (anionic) EOF. Subsequent addition of citrate-stabilized GNPs altered the direction of the EOF back to the cathode. The modification procedure using LPEI is shown in Scheme 6. LPEI and PDADMAC-modified channels provided more efficient separations of dopamine and epinephrine than poly(allylamine) hydro-chloride and chitosan. Moreover, the former polyelectrolytes yielded stable stationary phases in the pH range 3–11, which was not the case for chitosan. LPEI was ascertained to be the best choice and all following experiments were carried out on LPEI and GNPs-coated chips. The effects of buffer concentration, injection time and volume and separation voltage were studied in detail, linearity and LODs were determined. Repeatability of the retention times and the chip-to-chip reproducibility were also tested with very good results. It was demonstrated that GNPs greatly enhanced the sensitivity and efficiency of the separation.
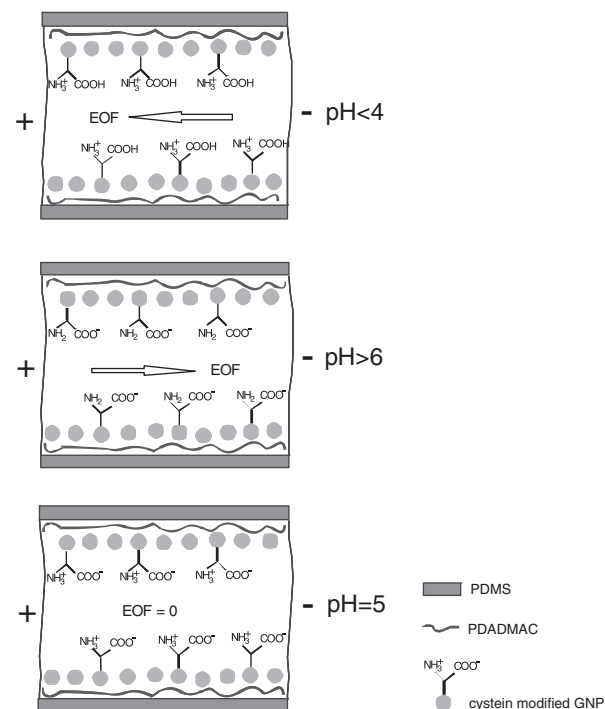
The same research group constructed also protein-coated PDMS microfluidic chips by the LBL technology [50]. Two slightly different protocols were utilized as shown in Scheme 7. The first approach, leading to the chitosan-GNPs-albumin microchip, was based on step-by-step modification of the PDMS surface with chitosan, citrate-stabilized GNPs (∼13 nm) and albumin. The other protein coating procedure avoided GNPs completely and the PDMS chip was succes-sively modified with proteins, forming lysozyme–albumin chip. Different entities immobilized stepwise on the PDMS surface influenced the EOF direction as shown in Scheme 7. PBS solution, pH 7, was used in the microchip fabrication and also as the mobile phase in separation experiments to prevent proteins from denaturing. Thus, when lysozyme (p*I* 11.0) was coated on the PDMS channel, EOF changed its direction from cathodic to anodic. Passing albumin solution through the lysozyme-coated chip led to the electrostatic adsorption of the opposite charged albumin (p*I* 4.7) on lysozyme surface, resulting in reversal of EOF again. The changes of EOF direction during the chitosan-GNPs-albu-min modification procedure were similar to that explained for lysozyme–albumin chip. Dopamine, epinephrine and several low-molecular mass pollutants were injected on PDMS microfuidic chip and an incomplete separation with significant peak tailing was observed. The migration time gradually increased from run to run indicating analyte adsorption and penetration to the PDMS surface. Thus, the surface modification was necessary to improve separation performance. The modification with multilayer film provi-ded significantly more stable EOF. It was realized that the

Scheme 7.

Scheme 8.

buffer concentration plays an important role and affects strongly the separation efficiency on the modified chips. Increasing the buffer concentration from 10 to 40 mmol/L, the corresponding resolution was also improved both on chitosan-GNPs-albumin and lysozyme–albumin chips and in contrast to the unmodified PDMS chip the baseline separation of all analytes was attained. The good repeatability and stability were obtained on both the protein-coated chips, also long-term stability was experimentally confirmed. Thus, the protein modification was successfully utilized to reduce analyte adsorption, improve separation efficiency and enhance EOF stability.
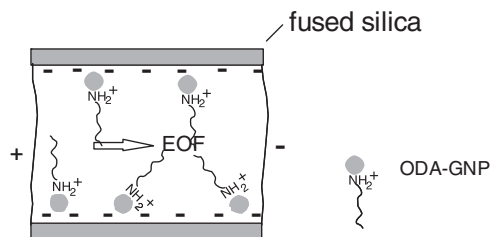
An EOF-switchable PDMS chip modified by GNPs with chemisorbed cysteine has been constructed using the LBL technology [70], see Scheme 8. The main binding forces between the successive multiple ionic layers were the electrostatic interactions. The surface of PDMS had negative charge at pH 7.0. The following layer of a quaternary ammonium polyelectrolyte (PDADMAC) carried a positive charge. Beside coulombic also hydrophobic interactions participated in the PDADMAC immobilization process. Cathodic EOF was changed to anodic. The next layer in the channel consisted of negatively charged citrate-stabilized GNPs (~15 nm) strongly adsorbed on the PDADMAC surface. Finally, cysteine creating the upper layer, was

chemisorbed through –SH group on the GNPs delivering the inner chip surface amphoteric character, where direction and extent of EOF was strongly pH dependent. EOF on the coated chip was studied in a wide pH range 3–12. At acidic conditions below 4.0, EOF was anodic, and at pH above 6.0, EOF was cathodic, at pH 5.0, the surface was neutral and EOF was suppressed. Good reversibility of EOF with pH was experimentally verified. The microchip coated with cysteine functionalized GNPs were rinsed with solutions of pH 3.0 and 12.0 and good stability of coating was confirmed. The separation characteristics of the stationary phase were evaluated with two groups of biomolecules, neurotransmitters (dopamine, epinephrine) and amino acids (arginine, histidine). The compounds were separated successfully on the coated microchip, and detection was electrochemical. Beside of applications in the separation field, the authors envisaged also the potential of the concept as microsensors to measure pH and other parameters and the possible usability of the device in biological and homeland security applications.

Recently, ODA-GNPs (Scheme 9) have been prepared and tested for OT-CEC separation of thiourea, naphthalene, biphenyl and several pharmaceutical steroid drugs [62]. The described preparation procedure was very simple. An FS capillary was just flushed with a solution of ODA-GNPs and because of the positive charges carried by the NPs, a strong electrostatic interaction between the negatively charged bare FS surface and modified GNPs took place. Hydrophobic interactions between siloxane groups present on the capillary surface and long aliphatic chains of ODA-GNPs participated in the adsorption process and the combination of both

Scheme 9.

interactions led to the very stable and robust stationary phase. Modification of the capillary by the ODA-GNPs suppressed EOF, but still the cathodic direction of the EOF was conserved in a broad pH range 3–11, and EOF was increasing with the increasing pH. Surprisingly, the ODA-GNPs layer remained stable even at pH 11, despite pK_a value of the adsorbed ODA is about 10.6. Sole hydrophobic interactions are probably responsible for such high pH resistance of the ODA-GNPs coating. The effect of ionic strength and methanol content in the mobile phase on chromatographic retention of the selected hydrophobic analytes was studied in detail. Stability of ODA-GNPs-modified capillary columns was much higher than that of capillaries directly coated by dodecanethiol GNPs *vide supra* [28].

4.3 HPLC

Application of GNPs in HPLC is much less than in CE and CEC. In fact, the only application of real gold particles, however not nano- but gold microparticles (GMPs) with average diameters 3.5 µm in HPLC, particularly in capillary LC, is the package of monodispersed spherical GMPs modified with *n*-octadecanethiol into a 100 µm id capillary column [64], see Scheme 10A. A mixture of small neutral organic compounds was used in capillary HPLC mode to test the stationary phase. Because of the low loading density of C18 on the GMPs, the retention factors of the analytes were relatively low in comparison with commercial octadecylsilane (ODS) FS-based capillary. Some shape selectivity for PAHs was found on this new stationary phase. The results showed that the new phase behaves basically as a reversed phase. Chemical stability of the column was excellent even at pH 12 for many hours, mechanical stability was also very high (at least 52 MPa). Neutral analytes provided symmetrical peaks with high efficiency, on the other hand for basic compounds serious tailing was found again.
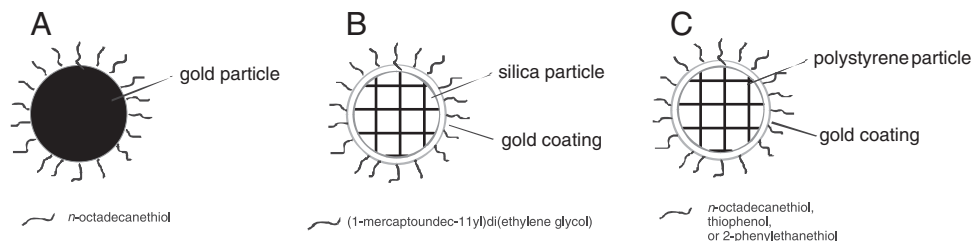
For the sake of the completeness, we briefly discuss here also the applications of SAM stationary phases on gold surfaces in the HPLC despite the truly GNPs were not used. The first work in this field was published by Ortiz *et al.* in 2001 [84] describing the synthesis and LC applications of SAM stationary phase composed of (1-mercaptoundec-11-yl)di(ethylene glycol) on gold-coated silica, see Scheme 10B. Next HPLC application of SAM approach was demonstrated by Kobayashi *et al.* in 2006 [63]. Gold-coated PSPs (5 µm)

were modified with three types of thiol compounds, specifically, *n*-octadecanethiol, thiophenol, and 2-phenylethanethiol, see Scheme 10C. The commercially available gold-coated PSPs (Piatech, Mie, Japan) were modified by the respective thio-compounds dissolved in ethanol. The comparison of chromatographic properties of aromates on the modified *versus* unmodified gold-coated PSPs and on a common ODS-silica sorbent led to several interesting findings. The newly prepared *n*-octadecanethiol-modified gold-coated PSPs phase demonstrated higher affinity to phenanthrene and anthracene than to small aromatics compared to the ODS column. The relationship between the retention factors and the ACN content in the mobile phase revealed higher sensitivity of the new column to the ACN content. All three new sorbents provided different chromatographic selectivity with respect to the ODS silica column. The main drawback of the thiol-modified gold-coated PSPs-based stationary phases was low separation efficiency and heavy peak tailing, both effects remain currently not fully explained.
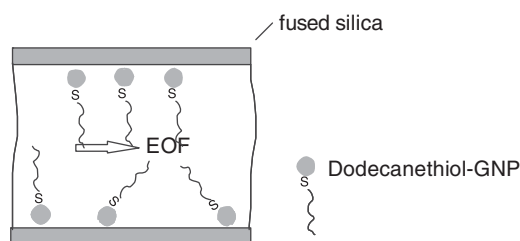
Modified GNPs were also employed for enantioselective recognition of chiral analytes, dansyl-D,L-norvaline [85]. In this case, BSA was covalently immobilized on silica supported GNPs. The prepared sorbent was slurry-packed into HPLC column. The effects of buffer composition, ionic strength, pH and methanol added into the mobile phase on resolution were studied in detail. Baseline separation of the enantiomers of the above dansyl-amino acid was reached under the optimized conditions.

4.4 GC

The first application of GNPs in capillary GC has been described by Gross *et al.* in 2003 [38]. They prepared monolayer-protected GNPs by covalent immobilization of dodecanethiol on the gold surface (Scheme 11). Deposition of the modified GNPs was obtained in a 2-m long, 530 µm id deactivated FS capillary. SEM analysis allowed for the estimation of 61-µm film thickness. The film was uniform in majority (~97%) of the inner capillary surface and no bare areas were revealed by SEM. The authors discussed the beneficial effect of the curvature of the modified gold core, which led to a larger population of core surface gold atoms in comparison with a flat gold surface, providing very fast mass transfer by diffusion. Since a wide range of thiol-based organic monolayers are available at these days, one can envisage GC stationary phases tuned for specific applications. GC technique can be also understood as an additional and complementary tool of characterization of the new synthesized GNPs. GC platform provides information on sorptive properties and the retention behavior of the modified GNPs and hence it represents a different approach than commonly used characterization techniques such as electron microscopies and thermochemical methods. In the discussed article, four classes of compounds (alkanes, aromatics, alcohols, and ketones) were studied on the

A

gold particle

~ *n*-octadecanethiol

B

silica particle

gold coating

~ (1-mercaptoundec-11yl)di(ethylene glycol)

C

polystyrene particle

gold coating

~ *n*-octadecanethiol,
thiophenol,
or 2-phenylethanethiol

Scheme 10.

fused silica

EOF

⬤ Dodecanethiol-GNP

Scheme 11.

dodecanethiol-GNPs-modified GC column. The retention behavior of the above analytes on the new stationary phase was compared with unmodified capillary and commercial AT-1 (Alltech) column. The chromatographic tests were carried out under the same conditions on all three columns to allow for a direct comparison of retention characteristics. AT-1 was intentionally chosen as the reference stationary phase because its similarity to the dodecanethiol-GNP-modified column. AT-1 is based on PDMS, the phase thickness was 100 μm id and length of the column was exactly the same as for the GNPs-modified capillary. The analytes had boiling points covering the range from 69 to 174°C. It was found that the analytes were not retained strictly upon the basis of their boiling points on dodecanethiol-GNPs-modified column. Retention order was based on the boiling points only within a homologous series of compounds. The AT-1 separation occurred in the same time frame as on dodecanethiol-GNP-modified column. However, the separation selectivity was slightly different indicating differences in retention mechanisms on the respective stationary phases. Beside the thermodynamic aspects, also kinetic behavior of the selected analytes on the columns was studied. Separation efficiency on the commercial and the GNPs-modified column was comparable and close to the maximal theoretically obtainable values. Further, the column robustness was tested and it was proved that the new stationary phases withstand hundreds of injections with little or no degradation. It was proved experimentally that the stability of the adsorbed layer of dodecanethiol-covered GNPs in the FS column is high in GC mode; this is contradictory to the low stability observed for the similarly prepared phase used in LC mode [28]. Finally, from TGA is was known that above ~175°C fast phase stripping takes place on dodecanethiol-GNPs-modified column. Consequently, temperatures applied in the study covered limited range only, specifically from 30 to 150°C. This can be definitely considered as relatively serious limitation with respect to PDMS stationary phases. However, on the other hand it should be mentioned that compounds with boiling point as high as 287°C were successfully analyzed on the new GNPs-based column.

More impressive GC separations were obtained by using dodecanethiol-GNPs-modified capillaries in a variety of the film thicknesses (10–60 nm) and with id from 530 to 100 μm [43, 44]. Very fast and high-efficient separations were reached with 100 μm id capillary and 10-nm film depth, six analytes were completely separated in less than 4 s. GNPs possessed favorable deposition properties and provided the large variety of potential organic monolayers, where the selection of the monolayer directs the chemical selectivity. The potential of this technology in micro-fabricated GC (μGC) was also tested extensively and successfully. A square cross-section (100 × 100 μm) dode-canethiol-GNPs-modified capillary was used as a model for a μGC system with angular cornered channels. SEM revealed that GNPs phase thickness was on average 15 nm on the capillary walls and not greater than 430 nm in the corners, however, from the point in the capillary where the phase is significantly higher to the center of the corner it is only 5 μm of the 100 μm wall length. Despite the thicker film in the capillary corner, chromatographic performance of the square capillaries was high, a minimum reduced plate height obtained with such system was very low, specifically 1.2 for octane, and up to seven analytes were well separated within 2 s. Such results are extremely promising for the further development of μGC. The versatility of the GNPs-modified FS capillaries was further found appealing also for applications in 2-D separation techniques such as GC × GC. Commercial polar phase PEG was connected (column 1) with GNPs-modified nonpolar dodecanethiol round capillary phase. The complementary stationary phases not only provided a larger separation space, but also gave chemical class information based on the separation pattern in 2-D space. Illustrative examples of the separation in GC × GC mode were provided (a class separation of alkanes/alkyl-benzene/ketones/alcohols and a gasoline "map"). The preparation of a polar 4-chlorobenzenethiol GNPs column turned out to be more difficult than dodecanethiol-GNPs phase and the synthetic procedure led to non-uniform deposition of the modifier onto the capillary walls. However, no bare spots were present along the length of the capillary allowing for the comparison of the retentive properties of the 4-chlorobenzenethiol and dodecanethiol-GNPs phase.

Initial experiments with the other polar phase were also carried out, 4-(trifluoromethy)-benzenethiol GNPs stationary phase appeared highly selective for polar analytes.

ODA-capped GNPs prepared by Qu *et al.* [62] were successfully applied not only in OT-CEC but also in GC [56]. Immobilization of ODA on the FS capillaries provided even more stable layer onto the inner wall, as positively charged ODA was adsorbed very tightly onto the negatively charged inner space of the capillary through electrostatic interaction. No degradation was observed even after about 1900 sample injections. Low-molecular-mass alcohols, aromates and esters served as the testing analytes. Similarly as for dodecanethiol-GNPs-modified capillaries, the temperature for the ODA-GNPs was relatively low. The mass loss started at 90°C, however, only a minute loss occurred at temperatures below 145°C and the analytes with a boiling point as high as 205°C were separated on the column. The minimum plate height, h_{min}, obtained for methylbenzoate was 5 that is slightly worse than the value obtained on dodecanethiol-GNPs-modified capillary. The ability to separate organic analytes was compared with nonpolar phase based on 100% PDMS, DB-1. The elution order of the studied compounds was the same on the both columns but the selectivity differed, which led to the significant improvement in resolution for certain critical pairs of compounds.

5 Application of GNPs for sample preconcentration and preseparation

5.1 Low-molecular-mass compounds

Very recently, a novel alternative approach to the extraction and preconcentration of PAHs from drinking water has been proposed by Wang *et al.* [66, 68]. The authors named the technique solid-phase nanoextraction and the method takes advantage of the strong affinity of PAHs to GNPs. Generally, 500 µL of contaminated water sample is mixed with 950 µL of a commercial solution of 20 nm GNPs in the first step. This is accompanied by the adsorption of PAHs on the surface of the GNPs. Next, centrifugation takes place, supernatant is discarded and the adsorbed analytes released from the GNPs by adding 2 µL of 1-pentanethiol and 48 µL of *n*-octane. The resulting solution is analyzed either by HPLC [66] or by laser-excited time-resolved Shpol'skii spectroscopy [68]. The optimization of experimental parameters led to the novel method with excellent analytical figures of merit, the high recovery and the low LODs. Moreover, as the entire extraction procedure consumes only about 50 µL of organic solvents per sample, the method can be considered as environmentally friendly. Preconcentration of aromatic analytes on GNPs was successfully used also for the improvement of the recovery and the LODs in the analysis of monohydroxy-PAHs in urine samples [67]. In this case, a typical SPE-HPLC procedure was profitably combined with solid-phase nanoextraction. In the course of experiments with SPE-HPLC, the authors found that the

main source of relatively low recovery of hydroxy-PAHs is the loss of analytes during evaporation of solvent following the SPE procedure. Thus, they substituted the evaporation step with a preconcentration of the analytes eluted from the SPE cartridge on GNPs. This modification of the method brought a significant improvement in performance of the overall procedure.

A selective enrichment of aminothiols was achieved by applying Tween 20-capped GNPs prior to CE coupled with LIF detection [59]. In contrast to citrate stabilized GNPs, Tween 20-capped GNPs exhibit the ability to be dispersed in a highly saline solution and the high extraction power for aminothiols. After extraction and centrifugation, 1 mmol/L thioglycolic acid was used to liberate aminothiols attached to the GNPs. In this way preconcentrated solutes were derivatized with *o*-phthaldialdehyde and determined by CE-LIF. The probe allowed for very significant sensitivity improvement for homocystein, glutathione, and γ-glutamycysteine.

Above-mentioned concept of Liu *et al.* [61], based on alkanethiol self-assembly and dithiol LBL technique comprising GNPs, was successfully applied for preconcentration of neutral steroids on GNPs-coated silica sorbent [57, 86]. The modified GNPs preparation procedure was similar to that described in [61]. It was found that multi-layer modified GNPs coated on smaller silica particles (5–20 µm) exhibited the best preconcentration efficiency for neutral steroids. Off-line concentrated solutes were subsequently determined either by GC [86] or by sweeping MEKC [57], where also an interesting clinical application was demonstrated. GNPs-coated silica SPE sorbent displayed superior clean-up efficiency toward the biological sample matrix, urine, with respect to a conventional C18-bonded silica SPE stationary phase. The results indicated that urinary proteins were effectively eliminated from the matrix in the preconcentration procedure probably due to their interactions with residual metal surfaces of the GNPs-coated silica sorbent. Modified GNPs-coated silica SPE material in combination with the optimized sweeping MEKC allowed for very efficient preconcentration, sample clean-up and the stacking of the analytes resulting in the 10^2–10^3-fold sensitivity enhancement for the analyzed steroids.

5.2 High-molecular-mass compounds

GNPs were utilized also in proteomics [52]. Protein enrichment is often necessary in protein studies and traditional precipitation methods are limited in the sample volume and protein concentration required to cause efficient precipitation. It was shown that GNPs can be applied to concentrate proteins from large volumes of urine (> 15 mL), in which the total protein concentration was less than 1.4 ppm. On the contrary, the commonly used trichloroacetic acid precipitation provided no enrichment for proteins in urine samples with volume above 2 mL or with protein concentration below 4 ppm. Moreover, GNPs-aggregated proteins can be directly applied to the gel electrophoresis for

GNPs-protein dissociation and following free protein separation as well as for the subsequent in-gel digestion and identification by MS. Citrate-stabilized GNPs aggregate a broad range of proteins in addition to those containing a high percentage of cysteine residues. Thus, the method is simple, efficient and very promising for enrichment of a broad range of proteins from highly dilute samples.

6 Concluding remarks

As the preceding content has documented, the separation sciences, especially the electromigration and chromatographic techniques, have achieved many advances employing GNPs as components of the separation systems. Although the application of GNPs is still in its infancy, their potential as multifunctional components to improve selectivity and separation efficiency has been already demonstrated.

The control of GNPs properties *via* modification of the gold surface with specific selectors provides opportunities to design unique separation systems. The ability to combine molecular functionality with the inherent properties of nanometer-sized entities makes an NP scaffold important tool for creation of complex functional systems.

Although the GNPs assisted techniques have not up to date provided a major break-through, the increasing role of gold and other NPs in separation science is evident. Undoubtedly, further research in the field is highly needed. The future work on the GNPs and their applications in separation science should cover several topics: (i) synthesis nearly (or completely) monodisperse modified NPs allowing at the same time faster mass transfer and possessing higher sample capacity, (ii) design and synthesis of highly selective interaction sites on the gold surface for on-line/off-line preconcentration and separation of the targeted analytes such as biomolecules and drugs, (iii) improvement in control over the integration of NPs into capillaries, (iv) improvement in stability and robustness of the prepared GNPs-modified capillaries, and (v) further development in the files of chip-based separations.

Finally, the authors would like to draw the readers' attention to the very recent applications of the modified GNPs for preconcentration and preseparation purposes, a highly promising utilization of the GNPs, where fast progress can be envisaged.

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