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Gold nanoparticles for specific extraction and enrichment of biomolecules and environmental pollutants

Abstract: Because of their high surface-to-volume ratio, easy surface modification, and simple synthesis methods, gold nanoparticles (AuNPs) are becoming an attractive material as an alternative to conventional solvent extraction and solid-phase extraction column for the selective extraction and enrichment of target analytes from a large sample volume. Through covalent bond formation (Au-S bonds), electrostatic attraction, hydrophobic adsorption, and molecular recognition, AuNPs have been applied successfully to the extraction/removal of a variety of compounds from biological fluids and environment waters, including thiol-containing compounds, peptides, proteins, heavy metal ions, polycyclic aromatic hydrocarbons, and melamine. This review summarizes the recent advances in properties, synthesis, and surface modification of AuNPs for the preconcentration of biomolecules and environmental pollutants.

Keywords: enrichment; extraction; gold nanoparticles.

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Introduction

Nanoparticles (NPs) and NP-based materials have a great impact on many fields, enabling researchers to develop different types of technologies (Katz and Willner 2004, Gijs et al. 2010, Talapin et al. 2010). Many researches have been accomplished in relation to the application of metal NPs in nanotechnology. Gold nanoparticles (AuNPs) are one of the most important materials because they provide distinct advantages, including size-dependent optical, electric, catalytic, and magnetic properties, great biocompatibility, and ease of chemical modification (Daniel and Astruc 2004, Rosi and Mirkin 2005). Moreover, AuNPs have a strong affinity for thiol-containing biomolecules, which are adsorbed spontaneously onto Au surfaces to generate self-assembled monolayers (Love et al. 2005). Numerous molecules, such as surfactants, polymers, proteins, and DNA, are shown to be spontaneously attached to the surface of AuNPs. Taken together, AuNPs are ideal for use in a variety of applications, for example, as biosensors, as drug carriers, as nanochemical devices, in cell imaging, and in separation science (Nilsson et al. 2007, Wilson 2008, Boisselier and Astruc 2009). Besides, because AuNPs possess a high surface area-to-volume ratio and high binding to thiol molecules, numerous studies have been devoted to understanding their impact on the field of extraction. The major goals in designing NPs as a preconcentrating probe are to control particle size, capping ligands, surface properties, and releasing agents.

This review details the recent development of gold-based nanomaterials in the extraction and enrichment of biomolecules and environmental pollutants, including thiol-containing compounds, proteins, peptides, indoleamines, heavy metal ions, polycyclic aromatic hydrocarbons (PAHs), and melamine. Also, we discuss the comparison of method characteristics. Table 1 summarizes the extraction and enrichment of biomolecules and environmental pollutants with various types of gold-based nanomaterials.

Workflow extraction

The extraction procedure can be divided into four steps (Huang and Chang 2006, Chang and Tseng 2010): (1) A large volume of target analytes is incubated with functionalized or bare AuNPs at an ambient temperature at an optimal time. (2) Target analytes are attached to the NP surface through molecular recognition, electrostatic attraction, hydrophobic adsorption, and covalent bonds. (3) Analyte-attached AuNPs are collected by centrifugation and washed with medium to reduce nonspecific binding. (4) Analytes adsorbed on the Au surface can be directly detected by matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) or NP-assisted laser desorption/ionization-time of flight-mass spectrometry (LDI-TOF-MS). In capillary

Material	Size	Analyte	Detection	LOD	References
Nile Red-absorbed AuNPs	32 nm	Cys, HCys, GSH	MALDI-TOF-MS	25–54 nM	Huang and Chang (2006)
Tween 20-AuNPs	13 nm	HCys, GSH, Glu-Cys	CE-LIF	79.8–4013.2 pM	Shen et al. (2009)
Tween 20-AuNPs	13 nm	GSH, Glu-Cys, PC_2, PC_3, PC_4	CE-LIF	0.1–6 pM	Shen et al. (2012)
Tween 20-AuNPs	13 nm	Removal of aminothiols	–	–	Huang and Tseng (2009)
FSN-AuNPs	13 nm	HCys	Fluorescence	180 nM	Lin et al. (2010b)
FSN-AuNPs	13 nm	HCys, di-HCys	Fluorescence	4.4–4.6 nM	Lai and Tseng (2012)
Tween 20-AuNPs	13 nm	GSH, HCys, Cys	CE-UV	28–554 nM	Li et al. (2009a)
Tween 20-AuNPs	13 nm	Cys, HCys, GSH, Cys-Gly, Glu-Cys	CE-UV	10–65 nM	Chang and Tseng (2010)
Citrate-capped AuNPs	11 nm	S-nitrosylated proteins	MALDI-TOF-MS	–	Faccenda et al. (2010)
Citrate-capped AuNPs	13 nm	Indoleamines	CE-LIF	4.1–366.0 pM	Li et al. (2009b)
Au@Magnetic particles	16 nm	Positive charged species	MALDI-TOF-MS	0.1 μM	Teng et al. (2004)
Tetraalkylammonium AuNPs	3–5 nm	Leu- and Met-enkephalin	AP-MALDI-MS	0.2 and 0.17 μM	Sudhir et al. (2005)
Citrate-capped AuNPs	13 nm	Proteins	Gel electrophoresis	–	Wang et al. (2006)
MM-capped AuNPs	–	Cytochorome *c*, lysozyme, myoglobin	MALDI-TOF-MS	13.2–40 fmol	Shastri et al. (2010)
Au-Fe_3O_4 composites	13 nm	Cytochrome *c*	MALDI-TOF-MS	–	Yu et al. (2010)
Fe_3O_4@C@Au particles	13 nm	Thrombin	MALDI-TOF-MS	0.36 nM	Zhang et al. (2012)
AuNP-coated silica NPs	180 μm	Hg^{2+}	AFS	180 pg l^{-1}	Leopold et al. (2009)
BMSAPD-capped AuNPs	20–60 nm	Metal ions	AAS	1.4–2.6 ng ml^{-1}	Karimipour et al. (2012)
Citrate-capped AuNPs	8.9 nm	Removal of Hg^{2+}	–	–	Ojea-Jimenez et al. (2012)
AuNP-Al_2O_3 particles	13 nm	Removal of mercury species	–	–	Lo et al. (2012)
Commercial AuNPs	20 nm	PAH	HPLC-fluorescence	0.9–58 ng l^{-1}	Wang and Campiglia (2008)
Commercial AuNPs	20 nm	Monohydoxy-PAH	HPLC-fluorescence	2–18 pg ml^{-1}	Wang et al. (2009a)
Commercial AuNPs	20 nm	PAH	LETRSS	0.8–88 ng ml^{-1}	Wang et al. (2009b)
Commercial AuNPs	20 nm	Benzo[*a*]pyrene	LETRSS	0.001 ng ml^{-1}	Wang and Campiglia (2010)
MUA-capped AuNPs	13 nm	Melamine	CE-UV	77 pM	Chang et al. (2010)

Table 1 Comparison of different types of AuNP-based nanomaterials.
AAS, atomic absorption spectrometry; AFS, atomic fluorescence spectrometry; AuNP, gold nanoparticle; BMSAPD, bis(4-methoxysalicylaldehyde)-1,2-phenylenediamine; CE-UV, capillary electrophoresis-ultraviolet; Cys, cysteine; di-HCys, disulfide homocystine; FSN, fluorosurfactant; Glu-Cys, glutamycysteine; GSH, glutathione; HCys, homocysteine; HPLC, high-performance liquid chromatography; LETRSS, laser-excited time-resolved Shpol'skii spectrometry; MALDI-TOF-MS, matrix-assisted laser desorption/ionization-time of flight-mass spectrometry; MM, (4-mercaptophenyliminomethyl)-2-methoxyphenol; MUA, 11-mercaptoundecanoic acid; PAH, polycyclic aromatic hydrocarbon.

electrophoresis (CE) analysis, the analytes are liberated from the Au surface through ligand-exchange reaction with a thiol reagent or a change in the pH of the solution. The analytes are isolated from NPs by centrifugation and then detected by CE.

Thiol-containing compounds

Low-molecular-weight aminothiols, including cysteine (Cys), homocysteine (HCys), glutathione (GSH), cysteinylglycine (Cys-Gly), and γ-glutamylcysteine (γ-GCS) have attracted considerable attention because they are capable of reflecting various clinical disorders, such as Alzheimer's disease and cardiovascular disease (Refsum et al. 1998, Cecchi et al. 1999). Up to now, significant attention has been given to the determination of aminothiols in biological fluids by using reversed phase-high-performance liquid chromatography (RP-HPLC) (McMenamin et al. 2009) and CE (Bayle et al. 2004, Carlucci and Tabucchi 2009). However, RP-HPLC is limited to the simultaneous determination of only four aminothiols in plasma (McMenamin et al. 2009). On the other hand, thiol-containing phytochelatins, with the common structure of $(\gamma\text{-glutamate-cysteine})_n$-Glycine ($PC_n$), mainly exist in plants and algae, and they act as chelating agents against the toxicity of heavy metal ions (Kawakami et al. 2006). For example, a high level of heavy metal ions in seawater may stimulate the production of phytochelatins in algae because phytochelatins can detoxify heavy metal ions via the coordination between heavy metal ions and the thiol group of phytochelatins (Chekmeneva et al. 2009). Current methods for the analysis of phytochelatins include RP-HPLC with inductively coupled plasma-mass spectrometry (ICP-MS) and RP-HPLC with fluorescence

detection. Huang and Chang (2006) prepared Nile Red-adsorbed gold NPs for extracting Cys, HCys, and GSH through the formation of Au-S bonds and applied them to the determination of Cys in plasma and GSH in red blood cells. Because Nile Red-adsorbed gold NPs were found to serve as LDI matrices, aminothiols attached to the NP surface were directly detected by NP-assisted LDI-TOF-MS. When 1 ml of sample solution was extracted with Nile Red-adsorbed gold NPs, the limits of detection (LODs) of Cys, HCys, and GSH were down to 25, 54, and 34 nM, respectively. This method provided an approximately 40-fold improvement in sensitivity. Shen et al. (2009) modified citrate-capped AuNPs with Tween 20 and used them for the selective extraction of HCys, GSH, and Glu-Cys. Tween 20 enables citrate-capped AuNPs to stabilize in a high-ionic-strength solution and minimize their nonspecific binding to proteins. After 8 ml of sample solution was extracted by using Tween 20-modified AuNPs (Tween 20-AuNPs), the extracted aminothiols were liberated from the NP surface through a ligand exchange reaction with thioglycollic acid. The released aminothiols reacted with *o*-phthalaldehyde (OPA) to form fluorescent products, which were detected by CE-laser induced fluorescence (CE-LIF). As a result, the use of Tween 20-AuNPs as concentrating probes provided approximately 11-, 282-, and 21-fold sensitivity improvement for HCys, GSH, and Glu-Cys, respectively, and the LODs of aminothiols at sub-pM levels. This present method was performed for the analysis of HCys, GSH, and Glu-Cys in the urine sample. However, the combination of Tween 20-AuNPs and OPA derivatization is insufficiently sensitive to detect GSH, Glu-Cys, and PC_2 in seawater (Shen et al. 2012). To overcome this problem, the same group introduced a simple method for the online concentration and separation of thiol-containing peptides in CE. When OPA-derivatized peptides were stacked by using poly(ethylene oxide) (PEO) solutions, the capillary was initially filled with background electrolyte. After a large volume of extracted aminothiols was injected, PEO entered a capillary with the help of the electroosmotic flow. OPA-derivatized peptides with negative charges migrate into neutral PEO solutions and slow down at the boundary between the sample zone and the PEO solution because of the viscosity difference. As a result, OPA-derivatized peptides were stacked and detected by CE-LIF. Compared with normal hydrodynamic injection without NP extraction, the combination of Tween 20-AuNPs, OPA derivatization, and CE stacking resulted in a more than 10,000-fold increase in sensitivity for CE-LIF detection of GSH, Glu-Cys, PC_2, PC_3, and PC_4, with the LODs of 0.1, 0.2, 2, 6, and 1 pM, respectively. This is the first use of CE to detect thiols dissolved in seawaters (Figure 1). In addition to the use of Tween 20-AuNPs as concentrating probes, Tween 20-AuNPs can remove aminothiols from urine and serum samples (Huang and Tseng 2009). Because OPA is a highly selective fluorescent reagent for HCys, GSH, Glu-Cys, and histidine, Huang and Tseng demonstrated a simple and selective method for detecting histidine by using Tween 20-AuNPs for the removal of aminothiols, followed by OPA derivatization.

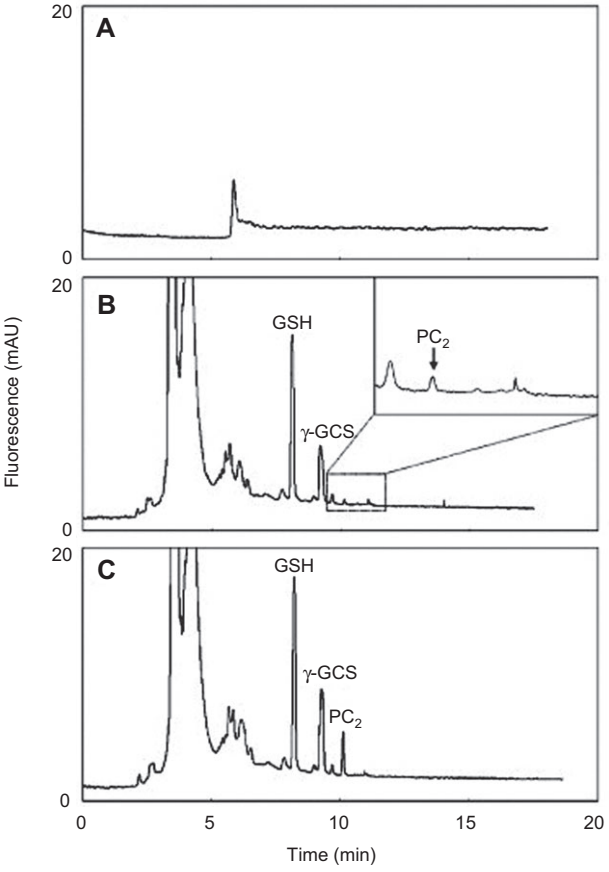

Figure 1 (A) Determination of dissolved thiols in seawater by CE-LIF without the combination of NP extraction and CE stacking. Extraction of (B) dissolved thiols and (C) dissolved thiols spiked with GSH (200 pM), γ-GCS (200 pM), and PC_2 (2000 pM) in seawater with Tween 20-AuNPs, followed by OPA derivatization and CE stacking. CE, capillary electrophoresis; CE-LIF, capillary electrophoresis-laser induced fluorescence; γ-GCS, γ-glutamylcysteine; GSH, glutathione; NP, nanoparticle; OPA, *o*-phthalaldehyde. (From the work of Shen and coworkers, with kind permission from Elsevier.)

The replacement of Tween 20 by a fluorosurfactant (FSN) can selectively capture Cys and HCys without the interferences of GSH, Cys-Gly, and Glu-Cys (Lu et al. 2007). Moreover, OPA reacts with HCys to form a highly fluorescent derivative in the absence of a nucleophile, but this is not true in the case of Cys (Benson and Hare 1975, Zuman 2004). These features are helpful to develop a sensitive and selective method for the analysis of HCys

in biological fluids. Lin et al. (2010b) described the use of FSN-modified AuNPs for selectively capturing HCys and Cys from urine samples. After centrifugation and washing, the addition of 2-mercapoethanol to the collected precipitates drove the liberation of HCys and Cys from the NP surface. The liberated HCys was selectively derivatized with OPA. The proposed system has the distinct advantages of high sensitivity (LOD=180 nM), greater linear range (0.6–40.0 μM), and excellent selectivity (over 100-fold for HCys over other aminothiols). Total HCys in plasma is composed of the major protein-bound HCys, homocystine (HCys–HCys disulfide; di-HCys), mixed disulfides containing a HCys residue, and trace amounts of reduced HCys. Lai and Tseng (2012) developed a convenient and sensitive method for the quantification of different forms of HCys in plasma by varying the order of disulfide reduction with tris(2-carboxyethyl)phosphine (TCEP). For example, to quantify protein-bound HCys, plasma samples were filtered by using centrifugal ultrafiltration. The obtained plasma proteins were treated with TCEP to release HCys. FSN-AuNPs were used for the selective extraction of the released HCys. The quantitative analysis of HCys in plasma proteins was accomplished after centrifuging, washing, thiol-liberated HCys, and OPA derivatization. This proposed method demonstrates that the combination of TCEP reduction, FSN-AuNPs extraction, and OPA derivatization is a reliable method to determine different forms of HCys.

Although CE-LIF provides a higher sensitivity and a better selectivity than does CE-UV absorbance, it usually requires derivatization of the analytes. To counter this drawback, Li et al. (2009a) prepared Tween 20-AuNPs for the selective enrichment of GSH, HCys, and Cys, prior to analysis by CE with UV detection. The extraction procedure includes NP aggregation formation, collection, and washing and the release of aminothiols. Although the LODs of GSH, HCys, and Cys were determined to be 28, 554, and 456 nM, respectively, the sensitivity of this method was sufficient to detect them in urine samples. To further demonstrate a real-world application of Tween 20-AuNPs extraction for a complexes mixture, Chang and Tseng (2010) described the use of Tween 20-AuNPs for capturing five aminothiols – Cys, HCys, GSH, Cys-Gly, and Glu-Cys – in plasma samples. Efficient separation of five aminothiols was successfully achieved by adding cationic polyelectrolyte, poly(diallyldimethylammonium chloride), to the background electrolyte (Yu and Tseng 2006, Lin et al. 2008). Compared with FSN- and Triton X-100-modified AuNPs, Tween 20-AuNPs had better aminothiol loading (Figure 2). LODs for the five aminothiols ranged from 10 to 65 nM. Because this extraction method

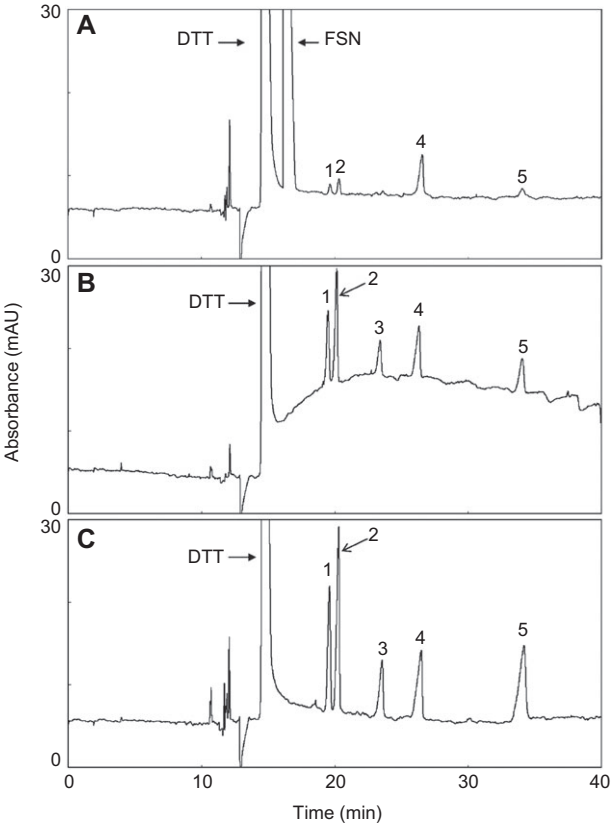

Figure 2 Extraction and enrichment of five aminothiols by AuNPs modified with (A) Zonyl FSN-100, (B) Triton X-100, and (C) Tween-20, followed by CE separation. Peak identified: 1, GSH (1 μM); 2, Glu-Cys (4 μM); 3, Cys (4 μM); 4, HCys (4 μM); 5, Cysgly (4 μM). AuNPs, gold nanoparticles; CE, capillary electrophoresis; Cys, cysteine; Glu-Cys, glutamycysteine; GSH, glutathione; HCys, homocysteine. (From the work of Chang and Tseng, with kind permission from American Chemical Society.)

can greatly reduce the matrix effect of the plasma sample, the concentrations of total and free aminothiols in plasma were quantified with an external calibration method.

Recently, Jia et al. (2009) disclosed that citrate-capped AuNPs can catalyze S-nitroso adducts with thiols, such as S-nitrosoalbumin and S-nitrosoglutathione, to generate nitric oxide. Meanwhile, this reaction induced the formation of Au-thiolate on the surface of AuNPs. Inspired by this result, Faccenda et al. (2010) used citrate-capped AuNPs to facilitate the identification of protein S-nitrosylation sites. Figure 3 illustrates the procedure for identifying S-nitrosylation and S-glutathionylation sites in proteins. Free thiols in proteins were initially alkylated with iodoacetamide. Proteins containing no thiols were digested to peptides. The addition of citrate-capped AuNPs to the digest led to the production of nitric oxide and the formation of AuNP-thiolate peptides. After centrifuging and washing, the peptides on the NP surface were

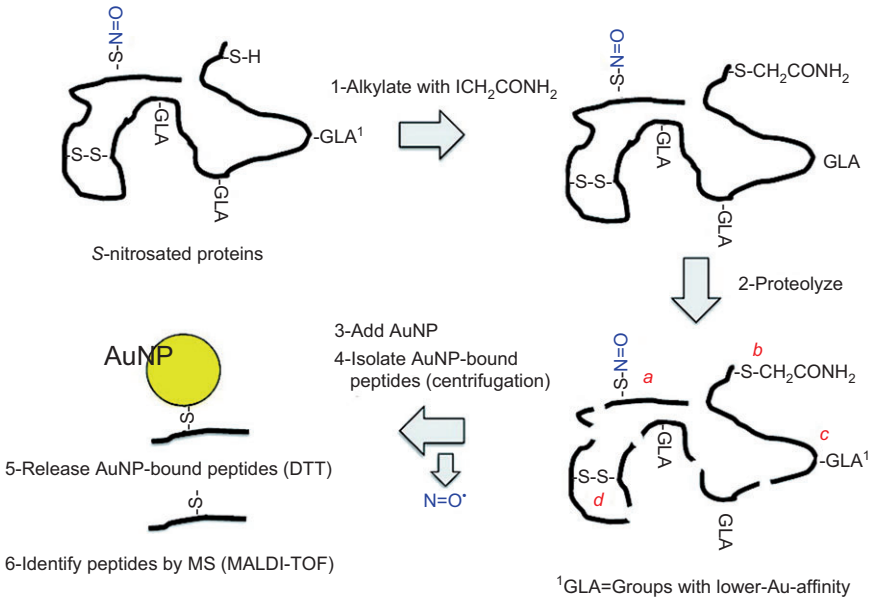

Figure 3 The combination of citrate-capped AuNPs and MALDI-TOF-MS for identifying S-nitrosylation sites in proteins. (From the work of Faccenda and coworkers, with kind permission from American Chemical Society.)

incubated with an excess of dithiothreitol (DTT) to release peptides. The released peptides were then identified by MALDI-TOF-MS. In addition to the above-mentioned biomolecules, Li et al. (2009b) reported that indoleamines, including tryptophan and its metabolites, were extracted and enriched with citrate-capped AuNPs via hydrophobic interactions and electrostatic attraction. Because indoleamines with an indole ring possess native fluorescence, the extracts were detected by CE coupled to laser-induced native fluorescence. The coupling of NP-based extraction to CE provided 48–4030-fold improvements in the LOD and showed successful analysis of tryptamine and serotonin in human urine.

Proteins and peptides

The successful analysis of proteins and peptides in biological samples relied on sample cleanup and enrichment because the proteins of interest are in relatively low concentration. Prior to two-dimensional gel electrophoresis and mass spectrometry, organic reagents such as trichloroacetic acid and acetonitrile are commonly mixed with crude samples to induce protein precipitation. Gold NPs have become another emerging material for the enrichment of proteins and peptides. Teng et al. (2004) prepared nanocomposites of AuNPs and magnetic particles via the sol-gel technique and demonstrated them to be efficient in capturing proteins and peptides through electrostatic attraction without interferences of surfactant and urea. The analytes adsorbed on the nanocomposites were characterized by MALDI-TOF-MS. Sudhir et al. (2005) combined tetraalkylammonium-functionalized AuNPs with single-drop microextraction for the selective preconcentration of peptides prior to the analysis by atmospheric pressure MALDI-MS. Peptides were extracted into a single drop of toluene through electrostatic attraction without the interferences of Triton X-100 and urea. Wang et al. (2006) evaluated the capabilities of citrate-capped AuNPs as a preconcentrating probe in the extraction and enrichment of proteins and compared them with the trichloroacetic acid precipitation method. Citrate-capped AuNPs were capable of enriching proteins from more than 15 ml of human urine, whereas the trichloroacetic acid precipitation method was ineffective in capturing proteins from a large sample volume (>2 ml). Shastri et al. (2010) synthesized (4-mercaptophenyliminomethyl)-2-methoxyphenol ligands and mixed them with $HAuCl_4$ in the presence of $NaBH_4$. In the single drop microextraction technique, the formed AuNPs served as extraction phases for peptide and protein. In the analysis of hydrophobic peptides, gramicidin D, this method achieved sensitivity improvement of up to 35-fold.

Because Fe_3O_4 NPs possess strong magnetism, they are readily isolated from sample solutions by applying

an external magnetic field. Yu et al. (2010) prepared poly(diallyldimethylammonium chloride)-coated Fe_3O_4 NPs and used them as templates for the self-assembly of citrate-capped AuNPs. The formed Au-Fe_3O_4 composites were shown to be effective for the selective extraction of Cys-containing peptides without centrifugation and ultrafiltration (Yu et al. 2010). Zhang et al. (2012) synthesized Au-Fe_3O_4 composites through multiple steps, including the formation and coating of Fe_3O_4 microspheres, the modification of poly(diallyldimethylammonium chloride), and the adsorption of citrate-capped AuNPs (Figure 4). The immobilization of Au-Fe_3O_4 composites with thrombin-binding aptamers was used for the selective extraction and enrichment of thrombin in human serum prior to the analysis by MALDI-TOF-MS. This method was capable of detecting thrombin at concentrations as low as 0.5 nM.

Environmental pollutants

The quantification of heavy metal ions in an aquatic ecosystem is of considerable interest because they pose severe risks to human health and the environment. For example, Pb^{2+} can inhibit brain development, while Hg^{2+} can damage the brain, heart, kidney, stomach, and intestines. Methods currently available for detecting heavy metal ions include flame atomic absorption spectrometry, inductively coupled plasma-mass spectrometry, and ICP-optical emission spectrometry. Because an Au surface exhibits a strong affinity for Hg^{2+}, Leopold et al. (2009) synthesized AuNP-coated silica particles and used them as a preconcentration probe for the determination of total dissolved mercury in seven different natural and effluent waters. No chemicals, such as chelating ligands and decomposition reagents, are required for the enrichment of dissolved Hg species. Moreover, the trapped mercury can be directly analyzed by using atomic fluorescence spectrometry without any eluent. The extraction of 7 ml of sample volume with AuNP-coated silica resulted in a low detection limit of 180 pg l^{-1}. Karimipour et al. (2012) loaded gold NPs on activated carbon and modified them with metal ion-chelating agents, bis(4-methoxysalicylaldehyde)-1,2-phenylenediamine. This sorbent possesses a strong affinity for Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{2+}, Pb^{2+}, and Zn^{2+} at pH 4.0. The extracted metal ions were released from the NP surface by using 4 M HNO_3. When the volumes of the initial solution and the eluting solution were 1600 and 6 ml, respectively, the sensitivity

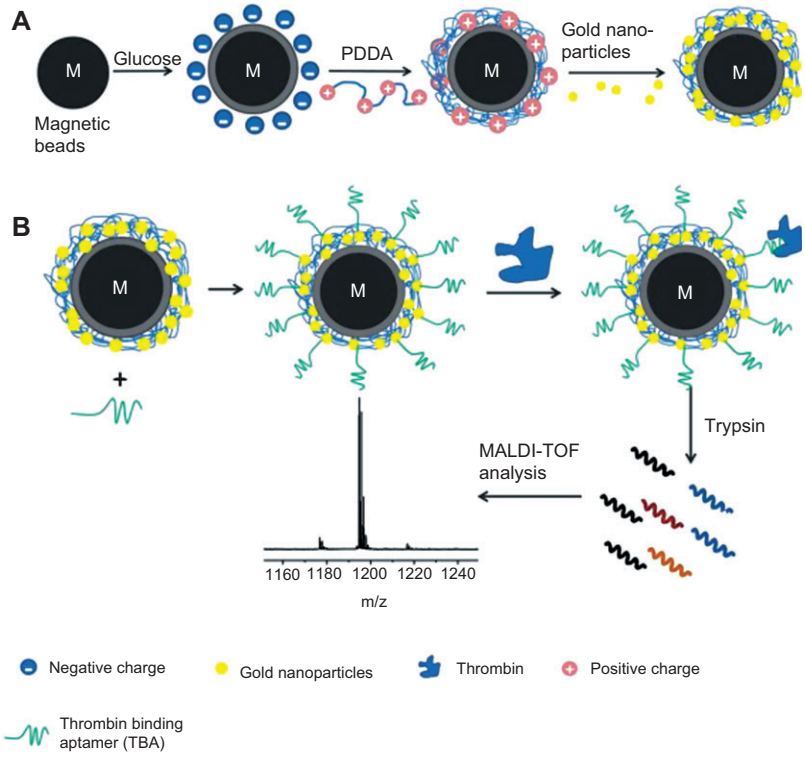

Figure 4 The combination of Au-Fe_3O_4 NP composites, aptpamer, and MALDI-TOF- MS for the analysis of thrombin. (From the work of Zhang and coworkers, with kind permission from Elsevier.)

was improved to 200-fold. Besides, the removal of heavy metal ions from water samples has attracted considerable attention because industrial wastewater often contains heavy metal ions. Ojea-Jimenez et al. (2012) reported that citrate-capped AuNPs were an effective sorbent material for removing Hg^{2+} from river water (Figure 5). AuNPs catalyzed the citrate-induced reduction of Hg^{2+} when citrate adsorbed onto the NP surface. The formed Hg(0) reacted with AuNPs to form a Hg-Au alloy on the NP surface (Lin et al. 2010a). When 6.6 nM AuNPs were added to 1 ml of 0.16 ppm Hg^{2+}, the removal efficiency of Hg^{2+} reached up to 100% after 24 h. On the basis of the interaction between AuNPs and Hg(0), Lo et al. (2012) synthesized the composites of AuNPs (13 nm) and Al_2O_3 particles (50–200 μm) as highly efficient for the removal of Hg^{2+}. Compared with AuNPs and Al_2O_3 particles, the AuNP-Al_2O_3 composites offered better removal efficiency for Hg^{2+}, methylmercury, dimethylmercury, and phenylmercury. The composites allowed effective removal of mercury species from lake water, groundwater, and seawater with efficiencies > 97%.

PAH originates from countless natural processes and human activities. The US Environmental Protection Agency (EPA) has identified a list of 16 chemicals as "Consent Decree" priority pollutants: benz[*a*]anthracene, pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, naphthalene, fluorene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene, indeno[1,2,3-*cd*]pyrene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluoranthene, chrysene, and benzo[*g*,*h*,*i*]perylene. Gas chromatography-mass spectrometry and HPLC-fluorescence spectroscopy are commonly used for the analysis of PAH. The extraction of PAH is a prerequisite for sample cleanup and preconcentration. Wang and Campiglia (2008) used citrate-capped AuNPs for the extraction and preconcentration of PAH, followed by HPLC with fluorescence detection. The extraction efficiency of PAH increased with a decrease in particle size because of an increase in the ratio of surface to volume. When a small volume (500 μl) of water sample was extracted with AuNPs, the LODs of 15 PAHs were down to 0.9–58 ng l^{-1}. The proposed method was also effective in enriching monohydroxy-PAH in urine samples (Wang et al. 2009a). The same group also combined the AuNP-based extraction and laser-excited time-resolved Shpol'skii spectroscopy (Wang et al. 2009b). PAH adsorbed on the surface of AuNPs was liberated on the addition of a mixture of 1-pentanethiol and *n*-octane. This developed method is capable of detecting 15 PAHs in drinking water without chromatographic separation. The entire procedure took less than 40 min per sample. By applying the proposed method, the LOD of benzo[*a*]pyrene was found to be 0.001 ng ml^{-1}, which is below the maximum permissible limits (0.2 ng ml^{-1}) of benzo[*a*]pyrene in drinking water permitted by the U.S. EPA (Wang and Campiglia 2010). It is well known that melamine with high nitrogen content has been added to milk products to boost its apparent protein content. Because melamine can bind to cyanuric acid, the formed complex may induce renal failure and even death in infants. Chang et al. (2010) demonstrated that the extraction of melamine was accomplished by using 11-mercaptoundecanoic acid-capped AuNPs via hydrogen bonding between the carboxylate groups of 11-mercaptoundecanoic acid and the amine groups of melamine. The extraction efficiency relied on the chain length of the mercaptoalkanoic acid and solution pH. The LOD was estimated to be 77 pM for melamine, with a linear range of 1–1000 nM.

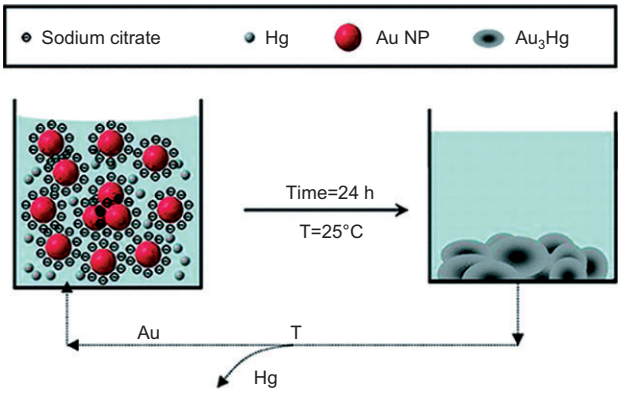

Figure 5 Citrate-capped AuNPs for the reduction and removal of Hg^{2+}. AuNPs, gold nanoparticles. (From the work of Ojea-Jimenez and coworkers, with kind permission of American Chemical Society.)

Conclusions

The successful examples described herein reveal that AuNPs are an appealing alternative for the selective extraction and enrichment of biomolecules and environmental pollutants because they are small, stable, and easy to prepare and bioconjugate. However, the collection of analyte-adsorbed AuNPs by centrifugation is time-consuming. In the opinion of the authors, the purification and enrichment of analytes of interest in complex mixtures could be accelerated by using a composite of AuNPs and magnetic NPs. It is possible to apply AuNPs to capture specific targets when they are modified with engineered DNA aptamers. Because a reduction in particle size results in an increase in the surface-to-volume ratio of nanomaterials, the extraction efficiency of analytes of interest may be remarkably improved by using ultrasmall AuNPs, such as organothiolate- and protein-stabilized gold nanoclusters (Negishi et al. 2005, Lin and Tseng 2010, Chen and

Tseng 2012). However, the collection of these gold nanoclusters is still challenging.

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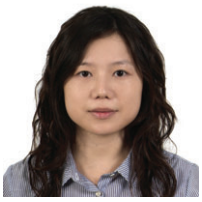

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