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Method development for speciation analysis of nanoparticle and ionic forms of gold in biological samples by high performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry☆

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ABSTRACT

A new method based on coupling high performance liquid chromatography (HPLC) to inductively coupled plasma mass spectrometry (ICP MS) has been developed for the speciation analysis of gold nanoparticles (AuNPs) and dissolved gold species (Au(III)) in biological samples. The column type, the composition and the flow rate of the mobile phase were carefully investigated in order to optimize the separation conditions. The usefulness of two polymeric reversed phase columns (PLRP-S with 100 nm and 400 nm pore size) to separate gold species were investigated for the first time. Under the optimal conditions (PLRP-S400 column, 10 mmol L^{-1} SDS and 5% methanol as the mobile phase, 0.5 mL min^{-1} flow rate), detection limits of 2.2 ng L^{-1} for Au(III), 2.8 ng L^{-1} for 10 nm AuNPs and 3.7 ng L^{-1} for 40 nm AuNPs were achieved. The accuracy of the method was proved by analysis of reference material RM 8011 (NIST) of gold nanoparticles of nominal diameter of 10 nm. The HPLC-ICP MS method has been successfully applied to the detection and size characterization of gold species in lysates of green algae *Acutodesmus obliquus*, typical representative of phytoplankton flora, incubated with 10 nm AuNPs or Au(III).

1. Introduction

Gold nanoparticles are widely used in many scientific, industrial and biomedical applications (e.g. electronics, sensors, catalysis, drug and gene delivery, optical bio-imaging of cells and tissues, contrast agent) [1]. Food supplements and cosmetics labelled to contain nanogold (e.g. lotions, skin creams, toothpastes, and soaps) are commercially available. That fact will certainly increase the exposure to this metal among humans and in the environment. The physicochemical properties of nanostructured materials due to their small size and large surface directly influence their fate and behaviour in environment and living organisms as well as their bioavailability and toxicity. These considerations are especially important for in vivo systems because nanoparticles can exhibit strong interactions with biological media. Their modification, transformation and metabolism depend on the pH, the ionic strength, the thermodynamic feasibility, the concentration of the species and also the redox conditions of the biological media. The term "nanometallomics" was proposed by Li et al. [2] for the research on the absorption, distribution, metabolism, excretion (ADME) behaviour of metal-related nanomaterials in biological systems. For such studies selective and sensitive methods for the determination of nanoparticles and products of their metabolism are essential. Moreover, the development of reliable analytical methods for speciation of gold in various natural materials (its forms and size of nanoparticles) might increase the knowledge on their transformation and interactions with natural components of organisms and environment, and in this way could reduce the potential risk of metal-related nanomaterials. The unique surface structure and reactivity of nanoparticles increase the possibility of their dissolution into ionic constituents (the smaller particle the higher solubility). Since nanoscale metal particles and metal ions may have independent or combined toxic effects, it is important to know whether the organism (i.e. plants and animals) is exposed to nanoparticles, ions or both. Therefore, analytical methods are required for the simultaneous determination of these forms [3]. Sample preparation procedures applied for NPs' characterization in size, concentration, composition and speciation in biological and environmental samples have been recently reviewed [4].

Inductively coupled plasma mass spectrometry (ICP MS) offers many benefits for the detection of trace amounts of gold, as wide

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calibration range and very low limit of detection could be obtained. In order to distinguish between nanoparticles and dissolved forms of gold, these fractions are usually separated by off-line methods such as centrifugal ultrafiltration, (ultra)centrifugation, dialysis, cloud point extraction and surfactant assisted dispersive liquid liquid microextraction [5–7]. The hyphenation of ICP MS with separation techniques, such as asymmetric flow field-flow fractionation (AF4), capillary electrophoresis (CE) and liquid chromatography (LC) have also been developed [6]. The drawbacks of AF4 technique are low recovery of nanoparticles and inability to separate dissolved forms of metal [8]. Extensive peak broadening for larger particles with a broad size distribution is a common problem in particle separation by CE technique [9].

So far only a few papers have been published on the application of liquid chromatography for the separation of nano and ionic forms of gold (Table 1). Size-exclusion chromatography (SEC) [10,11], hydrodynamic chromatography (HDC) [8,11] and high performance liquid chromatography (HPLC) have the potential for speciation analysis of gold. The main problem of using these modes is the adsorption of the NPs on the stationary phases, limiting their application for wide range of samples. Moreover, the eluent composition in SEC should be optimized for each type of sample [12]. HPLC in combination with ICP MS detection was applied for size determination of gold nanoparticles by Helfrich et al. [13,14]. The self-synthesised AuNPs standards and low-molecular Au(III) complexes were separated on Nucleosil C18 stationary phase with 100 nm pore size and 7 μm particle size. Although reversed-phase HPLC was used, the separation of NPs followed a size-exclusion mechanism. A good correlation between nanoparticle sizes determined by HPLC and by transmission electron microscopy (TEM) was observed [13]. The application of HPLC-ICP MS method provided a better understanding of the process of nanoparticle formation in solution [14]. The methodology based on the chromatographic separation developed by Helfrich et al. [13,14] was implemented to detect the

Table 1
Review of application of hyphenated LC-ICP MS technique for the characterization of gold nanoparticles.

Method	Separation conditions	Retention time (RT)	Analytical parameters	Sample	Ref.
HPLC-ICP MS	C: Nucleosil C18, 7 μm particle size, 100 nm pore size, 250 × 2 mm MP: 10 mM SDS, 1 mM NaH_2PO_4, 1 mM Na_2HPO_4, FR: 0.5 mL min^{-1}	4–5.5 min (AuNPs) 5.8 min (Au(III))	RSD < 1% (for RT, $n = 4$)	Self-synthesised AuNPs	13
	C: Nucleosil C18, 7 μm particle size, 30 nm and 100 nm pore size, 150 × 4.6 mm MP: 10 mM SDS, 1 mM NaH_2PO_4, 1 mM Na_2HPO_4, FR: 0.5 mL min^{-1}	5.3 min (25 nm AuNPs) 10.3 min (Au(III))	–	Self-synthesised AuNPs	14
	C: Nucleosil C18, 7 μm particle size, 100 nm pore size, 250 × 4.6 mm MP: 10 mM SDS,10 mM ammonium acetate (pH 6.8), FR: 0.5 mL min^{-1}	4.7 min (10 nm AuNPs) 5.7 min (Au(III))	RSD < 6% (peak area) Recovery: 91% ± 6% ($n = 3$)	Liver and spleen from Wistar rats	15
	C: Nucleosil C18, 7 μm particle size, 100 nm pore size, 250 × 4.6 mm MP: 10 mM SDS, 1 mM NaH_2PO_4, 1 mM Na_2HPO_4, FR: 0.5 mL min^{-1}	4.1 min (30 nm AuNPs) 4.7 min (10 nm AuNPs) 6.0 min (Au(III))	RSD: 1.8% (RT), 12% (peak area) LOD: 0.62 μg L^{-1} (AuNPs), 2.3 μg L^{-1} (Au(III)) Recovery: 66% (Au(III)), 95% (AuNPs)	HeLa cells (human cervical adenocarcinoma cell line)	16
	C: PLRP-S100, 8 μm particle size, 100 nm pore size, 250 × 4.6 mm MP: 10 mM SDS, 1% methanol, FR: 0.5 mL min^{-1}	3.8 min (10 nm AuNPs) 5.2 min (Au(III))	RSD: 1.9–5.6% (peak area), 0.4–0.6% (RT) LOD: 1.5 ng L^{-1} (Au(III)), 3.4 ng L^{-1} (10 nm AuNPs)	AuNPs standards	This work
	C: PLRP-S400, 8 μm particle size, 400 nm pore size, 150 × 4.6 mm MP: 10 mM SDS, 5% methanol, FR: 0.5 mL min^{-1}	3.1 min (40 nm AuNPs) 3.3 min (10 nm AuNPs) 3.7 min (Au(III))	RSD: 1.1–2.5% (peak area), 0.2–1.0% (RT) LOD: 2.2 ng L^{-1} (Au(III)), 2.8 ng L^{-1} (10 nm AuNPs), 3.7 ng L^{-1} (40 nm AuNPs) Recovery: 69.3–103.4% (tap water), 86.3–114.5% (algal cell lysate)	Tap water, *A. obliquus* cell lysate	This work
SEC-ICP MS	C: Venusil Durashell-NH_2, 50 nm and 100 nm pore size, 250 × 4.6 mm MP: 2% FL-70, 2 mM sodium thiosulfate (pH 9.5) FR: 0.5 mL min^{-1}		RSD: 0.1–0.6% (RT), 0.3–2.2% (peak area) LOD: 0.089 μg L^{-1} (Au(III)), 0.140 μg L^{-1} (5 nm AuNPs), 0.176 μg L^{-1} (10 nm AuNPs), 0.242 μg L^{-1} (30 nm AuNPs) Recovery: 93.0–98.4%	Environmental waters, serum	10
	C: Two MCX (sulfonated styrene/divinyl benzene copolymer), 10 μm particle size, 10^5 and 10^2 nm pore size, 300 × 8.0 mm MP: 0.5 mM Na_2HPO_4, 0.05% Triton X-100, 0.013% SDS, 0.05% formaldehyde, pH 7.5, FR: 0.25 mL min^{-1}		Recovery: 134 ± 10% (10 nm AuNPs), 108 ± 3% (30 nm AuNPs), 85 ± 2% (60 nm AuNPs)	AuNPs reference materials from NIST	11
HDC-ICP MS	C: Two PS-1(sulfonated styrene/divinyl benzene), 15 μm particle size (non-porous particles), 400 × 7.5 mm MP: 0.5 mM Na_2HPO_4, 0.05% Triton X-100, 0.013% SDS, 0.05% formaldehyde, pH 7.5, FR: 1 mL min^{-1}		Recovery: 124 ± 8% (10 nm AuNPs), 93 ± 4% (30 nm AuNPs), 98 ± 2% (60 nm AuNPs), 99 ± 2% (Au ionic)	AuNPs reference materials from NIST	11
	C: PL-PSDA Type-1 MP: 0.5 mM Na_2HPO_4, 0.05% Triton X-100, 0.013% SDS, 0.05% formaldehyde, pH 7.5, FR: 1.7 mL min^{-1}		LOD: 5 μg L^{-1} (AuNPs) Recovery: 77–96% (5 and 50 nm AuNPs)	AuNPs standards	8

C — column type, MP — mobile phase, FR — flow rate, HPLC — high performance liquid chromatography, SEC — size exclusion chromatography, HDC — hydrodynamic chromatography, SDS — sodium dodecyl sulfate, LOD — limit of detection, RSD — relative standard deviation.