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Analytical Note

Accurate determination of silver nanoparticles in animal tissues by inductively coupled plasma mass spectrometry ☆

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ABSTRACT

This study examined recoveries of silver determination in animal tissues after wet digestion by inductively coupled plasma mass spectrometry. The composition of the mineralization mixture for microwave assisted digestion was optimized and the best recoveries were obtained for mineralization with HNO_3 and addition of HCl promptly after digestion. The optimization was performed on model samples of chicken meat spiked with silver nanoparticles and a solution of ionic silver. Basic calculations of theoretical distribution of Ag among various silver-containing species were implemented and the results showed that most of the silver is in the form of soluble complexes $AgCl_2^-$ and $AgCl_3^{2-}$ for the optimized composition of the mineralization mixture. Three animal tissue certified reference materials were then analyzed to verify the trueness and precision of the results.

1. Introduction

Silver has long been valued as a precious metal, which is nowadays used mainly industrially [1]. Solutions of silver ions are used for example as disinfectants and microbicides. While many medical antimicrobial uses of silver have been supplanted by antibiotics, further research of its clinical potential continues [2]. Nanomaterials and nanotechnology are one of the fastest growing fields affecting virtually all aspects of our lives. We come across them in medicine as well as in food, textile, and automotive industries and many other sectors. The increased production of nanoparticles is reflected in a greater contact with them, which in turn brings questions about the safety of nanoparticles for living organisms, particularly in the case of long-term exposure. Because of their known antimicrobial properties [3], silver nanoparticles (AgNPs) are nowadays one of the most frequently used nanomaterials in consumer products [4]. Silver in the form of Ag^+ ions has toxic effects on many pathogens, including bacteria, viruses, and fungi [5]. Due to its relatively low toxicity in humans, silver has been used in various medical applications [6]. The antibacterial activity of AgNPs also depends on the Ag^+ ion, which is readily formed on the nanoparticle surface due to the oxidation of silver by oxygen. The antibacterial activity of AgNPs increases with a decreasing particle size, which has been associated with the increasing surface area-to-mass ratio [5]. Furthermore, silver nanoparticles are seen as a potential additive to animal feed, which might replace antibiotics [7,8]. Therefore, oral intake of silver nanoparticles is a relevant route of exposure for the consumers. Currently, many studies with experimental animals try to resolve safety and/or toxicity issues. Some of the experiments are focused on the study of the mechanism of AgNP actions on living organisms and on bio-distribution of nanoparticles in their internal organs and blood [9,10]. In the case of in vivo experiments, it is not only the toxicity of silver nanoparticles that is examined, but also their bio-distribution and accumulation in different organs of experimental animals [11,12].

A low concentration of silver can be determined by several instrumental techniques; nevertheless, only the application of inductively coupled plasma mass spectrometry (ICP-MS) will be discussed further. ICP-MS provides a very low limit of detection, and both Ag natural isotopes can be used. Possible spectral interferences on ^{107}Ag include ions of Ar–Ga, Ar–Zn, Ar–Ge, Zr–O, Sr–O, and Y–O. Isotope ^{109}Ag can be influenced by the presence of Ar–Ga, Ar–Ge, Nb–O, Zr–O, and Mo–O ions. Since the presence of zinc can be expected in significant concentration in real animal tissue samples, the interference of Ar–Zn ion can influence measurement. In this case, polyatomic interference of $Ar–Zn^+$ should be reduced by using collision/reaction cell in quadrupole ICP-MS or by using a high resolution ICP-MS instrument. Loeschner and his collaborators chose $^{107}Ag^+$ for their measurement on Agilent 7500ce because they had obtained a slightly lower limit of detection for AgNP suspension — 0.7 μg Ag mL^{-1} [13]. Quadrupole ICP-MS (X series from Thermo) was recently used for the development of a highly sensitive ICP-MS immunoassay by detecting silver deposited on the Au-NPs [14]. Under the optimum conditions, the ICP-MS calibration

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curves for $^{107}Ag^{+}$ with linear ranges of more than 5 orders of magnitude were routinely observed, and the limit of detection (3σ) was measured at 0.002 ng mL^{-1}. Xue and others [9] used ^{107}Ag for quantification of silver in mice on Thermo Elemental X7 ICP-MS. The limit of detection reported by them was 0.001 ng mL^{-1}. Recently, quantification of Ag^{+} release from nano-Ag by ICP-MS was also described [15]. Quadrupole ICP-MS (NexION300 from PerkinElmer) in a single particle mode was used for the simultaneous measurement of the dissolved and nano-Ag.

It is necessary to pay attention to the stability of Ag solutions. It is very critical because losses have frequently been described through sorption, the formation of colloids and precipitation. Container material, the pH, light and storage temperature play a decisive role for the stability of samples [16]. Low concentrations of silver are not stable in the absence of nitric acid [17]. West & Co. developed a flame atomic absorption spectrometry (FAAS) method for the determination of silver in water in the ppm to ppb range where EDTA is employed for the stabilization of the sample in order to prevent adsorption of silver onto container walls [18]. Great care must be taken when preparing solutions with Ag when chloride ions are present but their concentration is not high enough, since otherwise precipitation can easily take place in case their concentration is not high enough.

In most cases described in the literature, prior to analysis by atomic absorption spectrometry or ICP-MS, the biological samples undergo standard digestion in the mixture of nitric acid and hydrogen peroxide in a microwave mineralizer [9,12,19]. However, our preliminary experiments with chicken muscle spiked with Ag in the form of nanoparticles have shown that such an approach to sample digestion lowers recovery of silver to only about 70%. This agrees with the work of Loeschner et al. which mentions, without any details, that if no hydrochloric acid is added to the digestion mixture, recovery of silver is actually low [14]. Problems dealing with silver determination in animal tissue by AAS were described in the 1970s [20]. The authors tested several mineralization mixtures based on HNO_3 with $HClO_4$ for small and large samples (0.2, 10, and 50 g). Best recoveries were found for mineralization mixture consisting of HNO_3 + $HClO_4$ and addition of NH_4OH after mineralization, using about 5 mL of the acid mixture per gram of organic material present. In the research of distribution and accumulation of AgNP in rats' organs [21], their samples were weighted and carefully soaked in concentrated HNO_3 for 30 min; after the organic material was digested, the solution was heated at 90 °C to evaporate nitric acid and washed with 5 mL of HCl. The concentration of Ag was determined by ICP-MS (X series II, Thermo). Graphite furnace AAS determination of Ag in urine and blood after microwave digestion with 70% $HClO_4$ was published by Moradkhani & Co. [22]. The two last articles do not deal with recovery testing of the sample preparation.

The aim of this work was to optimize a procedure for the decomposition and determination of silver in animal tissues using ICP-MS, find suitable conditions for the determination of silver, and perform a method validation. By changing the content and the ratio of acids in the digestion mixture, recovery of the determination of Ag has significantly changed.

2. Materials and methods

2.1. Instruments

The silver concentration in all samples was determined by using an inductively coupled plasma mass spectrometer with a quadrupole mass analyzer and an octopole reaction system (7700×, Agilent Technologies, Japan); detailed working parameters are listed in Table 1. Isotope ^{107}Ag is the recommended mass when using the octopole reaction system for Agilent 7700 series ICP-MS. The concentration of Ag was quantified against an external calibration curve prepared from calibration water solution with certified concentration of Ag 1.000 ± 0.002 g L^{-1} (Analytika Ltd., Czech Republic). Solutions from 1 μg L^{-1} to 10 mg L^{-1} were prepared by dilution into 5% (v/v) nitric acid (Analpure, Analytika Ltd., Czech Republic). Rhodium (at concentration 100 μg L^{-1}) was used as an internal standard (mixed internal standard stock solution containing Rh, Analytika Ltd., Czech Republic).

Table 1
Working parameters of ICP-MS.

Parameter	Value
Isotope (m/z)	107
RF power (W)	1550
Sampling depth (mm)	8
Cool gas flow rate (L min^{-1})	14.95
Auxiliary gas flow rate (L min^{-1})	0.9
Nebulizer gas flow rate (L min^{-1})	1.09
He flow rate in ORS (mL min^{-1})	4.3
Concentric nebulizer flow (mL min^{-1})	0.4
Nebulizer pump speed (rps)	0.1
Chamber temperature (°C)	2
Dwell time per mass (s)	0.1
Number of replicates	3

For sample digestion (see below), a microwave digestion unit (MLS 1200 mega, Milestone, Italy) was used with the following time program: 2 min with 250 W; 2 min 0 W; 5 min 400 W; 2 min 0 W; 2 min 400 W; and, finally, 7 min 600 W.

2.2. Samples and certified reference materials (CRMs)

Chicken meat from a common slaughterhouse was stored in a freezer at −20 °C. Prior to the experiment, aliquot was thawed at room temperature.

The assessment of the trueness and precision of the measurement procedure was performed by the analysis of several certified reference materials: dogfish liver DOLT-4 (NRC-CNRC, Canada), with a certified value for silver of 0.93 ± 0.07 mg kg^{-1}; non-defatted lobster hepatopancreas LUST-1 (NRC-CNRC, Canada), with a certified value for silver of 0.580 ± 0.049 mg kg^{-1}; and oyster tissue SRM 1566b (NIST, USA), with a certified value for silver of 0.666 ± 0.009 mg kg^{-1}. Certified values for all used CRMs are presented along with their expanded uncertainties (k = 2).

2.3. Silver for spiking

For this study, we used silver in the form of nanoparticles (size 26 nm, polydispersity 0.156), prepared by a modified Tollens procedure [23], and a standard solution of ionic silver. Concentration of Ag in nanoparticle solution was 108 mg L^{-1} and it was determined by FAAS.

2.4. Sample preparation

Decomposition was performed in a microwave digestion system described above with concentrated HNO_3 (Analpure, Analytika Ltd., Czech Republic), or in a mixture with H_2O_2 (for trace analysis, Analytika Ltd., Czech Republic). Tissues were weighted directly into Teflon vessels. Prior to decomposition, samples were spiked directly in the vessels with a known concentration of silver in ionic form or as AgNPs. After the decomposition, 0.5–5 mL of HCl (Analpure, Analytika Ltd., Czech Republic) was added. Samples were then moved to 25 mL volumetric flasks and filled up with deionized water. Blanks were prepared for each digestion cycle with a mixture of digestion reagents (HNO_3, H_2O_2).

3. Results and discussion

3.1. Optimization of sample decomposition conditions

Due to a lack of literature data, great effort was given to the optimization of mineralization mixture for the decomposition of tissue samples. Chicken tissue samples were spiked with 0.05 and 0.1 mg L^{-1} of silver,