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Analysis of gold nanoparticles using ICP-MS-based hyphenated and complementary ESI-MS techniques

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ABSTRACT

Rapid growth in developments related to nanotechnology requires highly capable analytical techniques. They are urgently needed for the characterisation of the materials developed and manufactured as well as for the evaluation of their potential toxicity on ecological and biological systems. Moreover, reliable methods can help to understand the nanoparticle formation. In this work, we apply hyphenated techniques (liquid chromatography (LC) and gel electrophoresis (GE) coupled to inductively coupled plasma-mass spectrometry (ICP-MS)) to the characterisation of synthesised gold nanoparticles. The LC-ICP-MS approach is optimised for monitoring the formation of citrate-stabilised Au nanoparticles. The GE-ICP-MS methodology is used for the determination of Au^+/S^+-ratios in gold nanoparticles covered by mercaptosuccinic acid (MSA) by simultaneous ICP-MS detection of the sought elements. These ratios are a very valuable parameter for further characterisation of the nanoparticles. However, as ICP-MS does not provide any molecular information of the nanoparticle, electrospray ionisation-mass spectrometry (ESI-MS) is applied as complementary technique. The combined approach of ICP-MS and ESI-MS – well-known from speciation and metallomics studies – might be considered as an alternative powerful tool for future nanoparticle studies.

1. Introduction

Research on nanoparticles belongs nowadays to the most extensive areas in natural science. Nanoparticles have been recognised for their potential applications in various fields like biomedicine, electronics, optics, etc. This interest is attributed to their special properties effectively building a bridge between bulk material and atomic or molecular structures. The great variety in terms of chemical composition, size, form and surface modification offers immense possibilities in all areas where advanced material science is needed. Besides the great opportunities nanotechnology offers it has to be kept in mind that the use of nanoparticles might have a serious anthropogenic impact on environment and health, so that they also have to be considered as emerging contaminants [1,2].

Although not yet explicitly regarded as elemental species or within the concept of metallomics in the definition proposed by IUPAC [3,4], it was recently suggested to count metal- and semimetal-based nanoparticles as elemental species as well [5]. Indeed, nanoparticles are specific and unique elemental species due to their characteristic properties being different from either bulk material or low-molecular complexes and the difference in size is usually accompanied by different chemical composition. Therefore, in terms of environmental and health aspects they should be considered as elemental species in order to evaluate their bioavailability, their transport properties, and their specific cytotoxicity [6,7]. Various reviews have already pointed out the importance of determining their environmental and biological behaviour [1,2,8]. Considering the great diversity nanoparticles might have in terms of elemental composition, size, form, and surface modification, significant research work is depending on their reliable characterisation. As a consequence, analytical techniques are urgently required for the qualitative and moreover quantitative analysis of this emerging class of elemental species.

Hyphenated techniques with inductively coupled plasma-mass spectrometry (ICP-MS) were traditionally employed for elemental speciation studies [9] and are nowadays also directed to the determination of high-molecular elemental species in quantitative proteomics [10] and metallomics [11]. In the case of elemental nanoparticles their potential has been recognised in recent years [12,13], but this application field is still in its infancy regarding ICP-MS based hyphenated techniques. Field-flow fractionation

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(FFF) techniques in different modes can be considered as the most explored separation technique for nanoparticles with consequent detection by ICP-MS [14]. However, the separation power of this mild size-fractionating technique has been so far limited and especially nanoparticles below 10 nm diameter might be not accessible. More promising in terms of particle size separation are well-known chromatographic and/or electrophoretic techniques as reviewed recently by several authors [15–17]. In combination with ICP-MS detection the first approach was described by Helfrich et al. on the coupling of both, reversed-phase liquid chromatography and continuous elution gel electrophoresis, for size determination (5–20 nm) of synthesised gold nanoparticles [18]. Recently, hydrodynamic liquid chromatography was used for the study of different metallic nanoparticles in natural environment [19]. It can be expected that more studies will be directed to the development of ICP-MS based hyphenated techniques for the study of metal- and semimetal-based nanoparticles.

In this work, we present initial results on the use of ICP-MS hyphenated techniques (particularly liquid chromatography [20] and continuous elution gel electrophoresis coupled to ICP-MS [21]) as an alternative and powerful tool for the characterisation of nanoparticles. Liquid chromatography was in particular interesting for observing the genesis of nanoparticle formation. Additionally, on the example of laboratory-made Au nano-sized particles covered by sulphur-containing ligands the electrophoretically separated species will be characterised by the determination of the corresponding metal–sulphur ratios. These experimental results will be compared to the theoretically expected values. These initial experiments shall demonstrate the usefulness of ICP-MS based hyphenated techniques for the analysis and characterisation of nanoparticles. Furthermore, ESI-MS was exemplarily employed as complementary technique for the molecular characterisation of the ligand bound to the surface of the nanoparticles.

2. Experimental

2.1. HPLC coupled to ICP-MS

For HPLC separation different reversed-phase columns were connected to the ICP-MS via a cross-flow nebuliser and a Scott spray chamber (both obtained from AHF Analysentechnik, Tübingen, Germany). The mobile phase consisted of a phosphate buffer (1 mmol L^{-1} NaH_2PO_4, 1 mmol L^{-1} Na_2HPO_4, pH = 7.3) with the addition of 10 mmol L^{-1} SDS (sodium dodecyl sulphate, all Sigma–Aldrich, Deisenhofen, Germany) and delivered at a flow rate of 0.5 mL min^{-1} (S1121 solvent delivery system pump and a S8110 gradient mixer (both Sykam, Fürstenfeldbrück, Germany). All connections were made of PEEK and the samples were introduced by means of a metal-free Rheodyne 9725i injection valve (Phenomenex, Aschaffenburg, Germany) equipped with a 20 μL PEEK sample loop. Two C18 columns were used for these studies, which differed in terms of their pore size: Nucleosil 7 μm particle size, 300 Å and 1000 Å pore size, 150 mm × 4.6 mm ID both equipped with security guard cartridge Widepore 4 mm × 2 mm ID (both from Phenomenex). Before injection the samples were filtered through 0.2 μm filters (membraPure, Bodenheim, Germany, Membrex 18 PET unsterile diameter 18 mm). Although this filtration step might have an impact on the quantitative recovery of the nanoparticles, their composition in terms of the size range did not change.

2.2. Gel electrophoresis coupled to ICP-MS

The coupling of the gel electrophoretic system (Mini Prep Cell with high voltage supply PowerPac 3000, BioRad Laboratories, Munich, Germany) with the ELEMENT2 ICP-MS was realised as described in detail in earlier work [22]. The ICP-MS was operated in medium resolution mode ($m/\Delta m = 4000$) in order to resolve the spectral interferences (mainly $^{16}O^{16}O^+$) for $^{32}S^+$ detection. Further instrumental parameter are summarised in Table 1.

Table 1
Instrumental parameters of the ELEMENT2 ICP-MS.

ICP system	
Instrument	Element 2
RF power	1300 W
Auxiliary gas flow	0.8 L min^{-1}
Coolant gas flow	16 L min^{-1}
Nebulizer gas flow	0.8 L min^{-1}
Sampler cone	Pt, 1.0 mm orifice
Skimmer cone	Pt, 0.7 mm orifice
Dwell time per isotope	100 ms
Isotopes monitored	$^{32}S^+$, $^{197}Au^+$
Mass resolving power	4000 (medium)

Various experimental parameters (voltage, buffer composition and concentration, gel length and concentration) were optimised for the electrophoretic separation of the nanoparticles. Depending on the nanoparticles analysed the gel composition varied between 2.0 and 2.4% (w/w) SeaKam®LE agarose (Biozym, Hessisch Oldendorf, Germany), voltage was usually set at 400 V, and the electrode buffers contained 1 mmol L^{-1} NaH_2PO_4 and 1 mmol L^{-1} Na_2HPO_4 (pH = 7.3). The elution buffer additionally contained 10 ng g^{-1} Rh as internal standard and was transferred to the cross-flow nebulizer at 0.3 mL min^{-1}. Gel dimensions were kept constant (80 mm × 1.2 mm ID) and the injected sample volume was 1 μL.

2.3. ESI-MS analysis of the nanoparticles

As complementary technique ESI-MS was employed for the characterisation of the nanoparticles. The nanoparticles were obtained by collecting fractions after gel electrophoretic separation. The samples were usually diluted 1:1 in methanol and injected into the ESI-MS by means of syringe pump. A LCQ ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) was employed in the negative mode at a capillary voltage of −3.5 V. Collision-induced dissociation (CID) took place in the ion trap for a selected $[M-H]^-$ precursor ion and helium was used as the collision gas. Relative collision energy for ion trap fragmentation was adjusted to 30%.

2.4. Synthesis of Au nanoparticles

Besides two commercially available gold colloid solutions (nominal 5 and 20 nm particle size monodisperse, Sigma–Aldrich) citrate reduced gold nanoparticles (modified after Turkevich et al. [23]) and particles modified with mercaptosuccinic acid (MSA) were synthesised after Brust et al. [24].

For studying the formation of citrate-stabilized Au nanoparticles the reaction was carried out at room temperature in order to slow down the reaction. For this purpose, a solution of 1 mg L^{-1} Au standard (Alfa Aesar, Karlsruhe, Germany) was prepared in 5 mmol L^{-1} trisodium citrate dihydrate (>99%, Sigma–Aldrich) and 15 mmol L^{-1} SDS (pH = 6.0). The progress of the particle formation was monitored every 30 min by removing a few μL from the stirring solution with immediate injection into the LC system.

Typically, the formation of MSA-stabilised Au particles [25] was carried out using a high molar S/Au-ratio of ~5:1. For this, 40.4 mg hydrogen tetrachloroaurate(III) ($HAuCl_4 \cdot 3H_2O$, Sigma–Aldrich) was dissolved in 20 mL methanol and 20 mL of 76.9 mg MSA in 20 mL methanol was added. After stirring for 5 min 10 mL of 38.1 mg

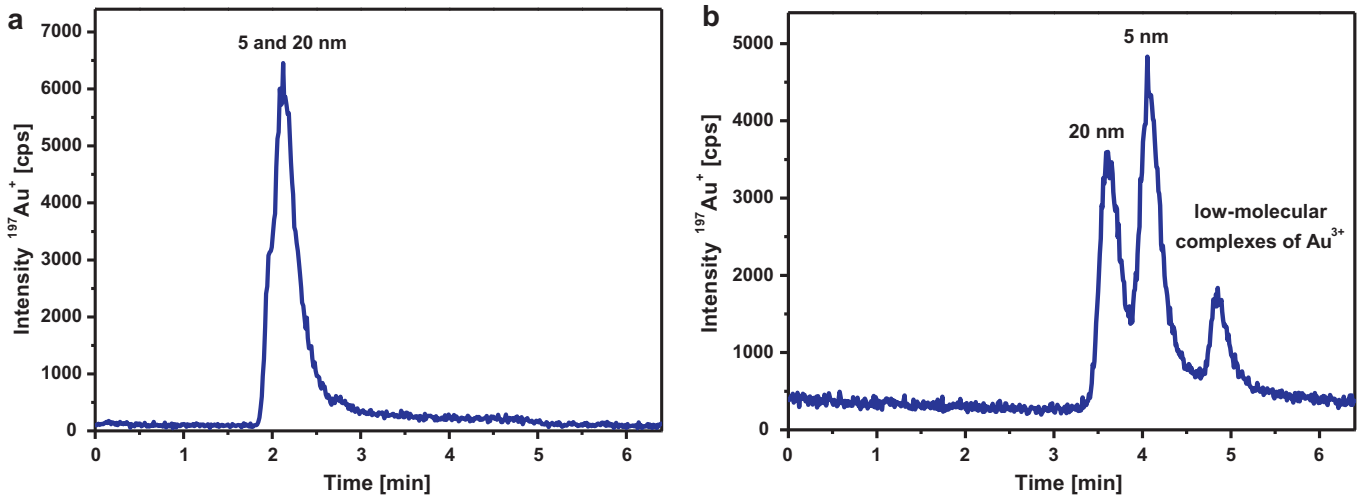

Fig. 1. $^{197}Au^{+}$-chromatograms of a mixture containing 5 and 20 nm Au NP standards using a C18 column (a) with 300 Å pore size, and (b) 1000 Å pore size.

sodium tetraborohydride (98%, Acros Organics, Geel, Belgium) was dropwise added to the solution. After 1 h stirring the suspension was centrifuged at 4000 rpm for 30 min followed by two washing steps using 20% (v/v) water/methanol, and then twice with pure methanol in order to remove salts and excess of MSA. Finally, the nanoparticle sample was dried under vacuum at room temperature. For further investigations an aliquot was dissolved in water or in the electrode buffer solution.

3. Results and discussion

3.1. HPLC separations of Au nanoparticles with subsequent ICP-MS detection

First investigations were aiming the application of reversed-phase HPLC for the separation of citrate-stabilized Au nanoparticles. Commercially available formulations (5 and 20 nm particle size, resp.) were used for the chromatographic optimisation. At the beginning a phosphate buffer served as mobile phase, but strong interactions between the stationary phase and the nanoparticles were observed resulting in extremely low column recoveries. Following the suggestions of adding surface-active substances like SDS in both, chromatography and electrophoresis, resulted in a significantly improved species recovery [26,27]. Therefore, an optimum concentration of 10 mmol L^{-1} SDS was kept throughout the chromatographic work.

Although using reversed-phase HPLC the separation of nanoparticles followed a size-exclusion mechanism [18]. This can be easily demonstrated by choosing C18 material with differing pore size. As demonstrated in Fig. 1a the application of 300 Å pore size did not allow the separation of 5 and 20 nm gold colloids under the chosen conditions, whereas these two different-sized nanoparticles could be discriminated using the chromatographic material with the increased pore size (1000 Å, Fig. 1b). The smaller pore size did not permit the nanoparticles entering, which is also documented by the short elution time (~2 min) close to the dead volume. Obviously, the hydrodynamic diameters of these nanoparticles were, first of all, similar and too large for this type of chromatographic material. As a consequence, the stationary phase with 1000 Å pore size used throughout this work, which permitted the separation of different-sized nanoparticles following a size-exclusion mechanism. Furthermore, these working conditions allowed the detection of low-molecular complexes of Au^{3+}, which might be important in future experiments, e.g., related to toxicity studies, in which the role of metal ions not bound to nanoparticles can be examined.

In this work we applied the chromatographic method for evaluating exemplarily the process of nanoparticle formation. Several investigations are directed into the basics of these processes for gaining a better understanding with the aim to improve the control of the syntheses, for instance, in terms of narrow size distributions or shape [28–31].

The reaction rates of nanoparticle formation using Turkevich method [23] are usually quite high in comparison to the time required for a chromatographic run. Therefore, the reaction for the formation of the citrate-stabilized Au nanoparticles was carried out at room temperature in order to decrease the reaction rate. As described in the experimental part after initialising the reaction a small aliquot was taken every 30 min and directly injected into the chromatographic systems. The resulting chromatograms are presented in Fig. 2 (in contrast to Fig. 1 the pH of the mobile phase was adjusted to 6.0). Directly at the beginning a peak at 9 min could be observed besides the precursor peak ($[AuCl_4]^-$) at 10.3 min, whereas the predicted nanoparticle (5.3 min with a nominal particle size of ~25 nm, which was confirmed by TEM) could be first detected after 30 min reaction time to a very low extent. With time the precursor disappeared completely and the amount of the final product (the citrate-stabilized nanoparticles) increased, while the intermediate passed a maximum in abundance between 0.5 and 1 h reaction time. These findings supported the formation process via an intermediate form as recently reviewed in the literature [28]. However, whether more than one intermediate was involved in the nanoparticle formation could not be answered. Nevertheless, the proposed LC-based approach might serve as alternative, relatively simple technique for monitoring reactions of nanoparticle formation provided that the reaction rates meet the time frame of a chromatographic run.

Although SDS as chromatographic modifier permitted the separation of the sought nanoparticles, its use was accompanied by two important drawbacks: (i) in the case of studying nanoparticles containing sulphur reagents as surface modification the potential simultaneous detection of $^{197}Au^{+}$ and $^{32}S^{+}$ could not be realised due to the high background level of sulphur, and (ii) further ESI-MS studies for the molecular characterisation of these species was strongly hampered by the presence of SDS. Therefore, an alternative hyphenated technique, namely gel electrophoresis, was applied in order to complement the above shown developments. The aims were to evaluate the Au/S-ratios in the nanoparticle fractions and to gain molecular information of the surface modification by ESI-MS.

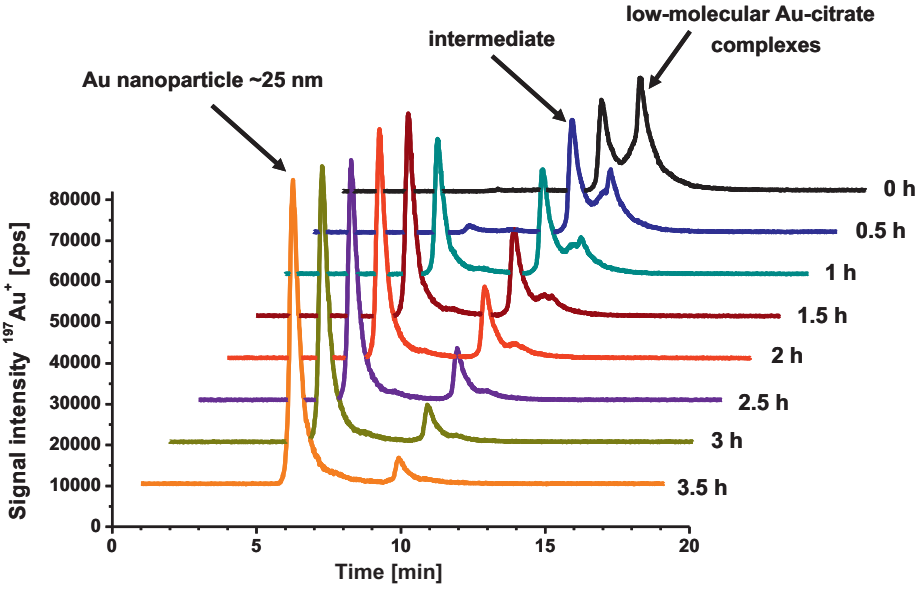

Fig. 2. Formation of citrate-stabilised Au nanoparticles monitored with HPLC-ICP-MS.

3.2. Separation of Au nanoparticles using GE with subsequent ICP-MS detection

Gel electrophoresis showed already great potential for the separation of Au nanoparticles of different size [18,32] or even shape [32]. For the realisation of gel electrophoresis combined with ICP-MS detection two principles of coupling are nowadays known: (i) the conventional slab gel electrophoresis with subsequent laser-ablation and ICP-MS first developed by Neilsen et al. [33], and (ii) the on-line coupling of GE and ICP-MS as developed in our laboratories [21]. The latter one was used within this work as it provides an easier experimental set-up.

In earlier work the addition of SDS as modifier in the electrode buffers provided a sufficient separation of citrate- and MSA-stabilized Au nanoparticles [18]. As the aim of this study was the analysis of the Au/S-ratios in the nanoparticle fractions, the separating conditions needed to be modified. After careful optimisation the applied buffer solutions contained uniquely NaH_2PO_4 and Na_2HPO_4 (both 1 mol L^{-1}). These conditions were applied to the analysis of the synthesized MSA-stabilized nanoparticles as described in Section 2 (Fig. 3). Both monitored isotopes ($^{32}S^+$ and $^{197}Au^+$) showed a dominant signal at 7.3 min and a smaller one at 5.3 min. In order to get a deeper insight into the molecular composition of the eluting fractions, the simultaneous quantification of the two elements was chosen as possibility for the determination of the Au/S-ratios in the nanoparticles. These ratios represent an important property of a nanoparticle, which further characterize the sought nanoparticle. For the determination of the elemental ratio we followed a procedure used for the determination of the phosphorylation degree in proteins via the phosphorous/sulphur ratio [34]. In this case, the detection sensitivities for the two isotopes were determined by the use of two standard solutions ($AuCl_4^-$ and SO_4^{2-}), injected into the GE-ICP-MS system at different concentrations. These data were applied to calculate the Au and S concentration of the eluting nanoparticle fractions (for both, calibration and analysis in the nanoparticle fractions, peak areas were determined). The results from these analyses were molar Au/S-ratios of 1.19:1 (peak at 5.3 min) and 1.33:1 (7.3 min) with RSDs typically below 5% ($N = 3$). First of all, the increase of the values corresponded with the elution order directed by the size of the nanoparticles. Following e.g., the model of an ideal cluster containing icosahedral packing, a nanoparticle consists of one core atom (in this case Au) with surrounding shells, in each nth shell containing $10n^2 + 2$ atoms (or on the surface ions) [35]. Provided that each Au^+ ion on the surface is "protected" by one ligand (in this case MSA) the number of ligand molecules is equal to that number of outer shell Au^+ ions. Thus, the ratio between the number of core atoms/ions to the number of ligand molecules raises with increasing nanoparticle size, e.g., MSA-protected Au_{13} clusters would result in a Au/S-ratio of 1.08:1 and MSA-protected Au_{55} clusters in a ratio of 1.33:1. Taking these theoretical considerations into account the obtained values (1.19 and 1.31, resp.) could be interpreted as follows: Both values represented small-sized nanoparticles, which also correlated with the conditions of the synthesis (the expected particle sizes were in the range of about 1 nm due to the chosen molar ratio between the precursors $HAuCl_4$ and MSA of 1:5). The Au/S-ratio of 1.19 for the peak eluting at 5.3 min (Fig. 3) indicated that the nanoparticle composition was found in between the Au_{13}- and the Au_{55}-cluster. Further investigations are still necessary to clarify its exact molecular composition with respect to the number of bound MSA molecules. The second peak at 7.3 min showed a value of 1.31, which was in excellent agreement with the ratio expected for an ideal Au_{55}-cluster with maximum coverage of 42 MSA molecules. However, a certain proof could not be obtained from these data obtained by ICP-MS. As a consequence, ESI-MS as a complementary technique was applied in order to more detailed information on the molecular composition of this Au nanoparticle.

3.3. ESI-MS analysis of the fractionated Au nanoparticles

Mass spectrometry, especially ESI- and MALDI-MS, is well-known as a very versatile tool for the characterisation of nanoparticles [36–40]. Its main advantage over other techniques is that entire clusters can be determined and identified with respect to their chemical composition provided that artefacts or degradation processes during the ionisation can be excluded. In order to explore the possibility of ESI-MS in the present, the focus was set on the analysis of the most abundant nanoparticle fraction eluting at 7.3 min (Fig. 3). For this, the fraction was collected, diluted 1:1 with methanol and introduced into the electrospray source by the use of a syringe pump. A typical mass spectrum recorded in the negative mode is shown in Fig. 4. The conditions used for these measurements did not provide any signal for entire nanoparticles,

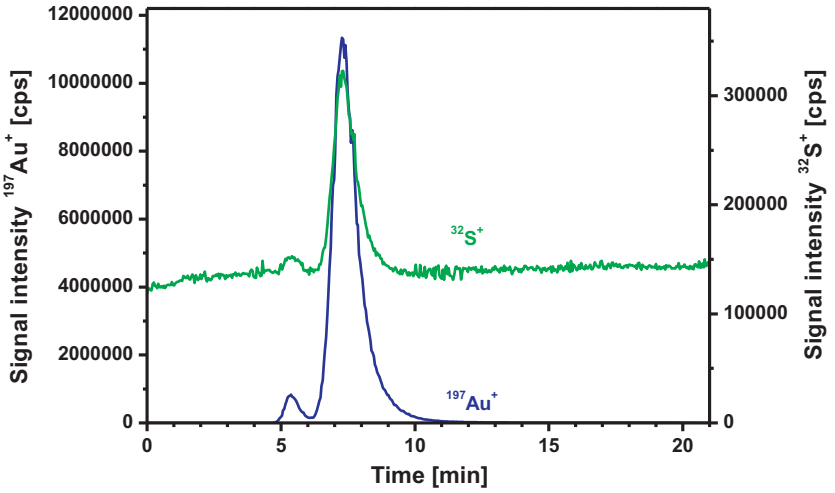

Fig. 3. Electropherogram of the MSA-stabilized Au nanoparticles.

but fragments of the clusters were probably derived from the particle's surface [41] and were observed with significant abundances. However, the most intense signal was detected for $[Au(MSA)_2]^-$ at m/z 495, but more interestingly, abundant negative ions could be observed for $[Au_n(MSA)_n]$ species (n = 2–4) and their corresponding sodium adducts. Comparative ESI-MS measurements of a mixture containing $[AuCl_4]^-$ and MSA (data not shown) resulted in a most abundant signal at m/z 495 as well, but any high-molecular clusters of Au and MSA could not be detected. This might indicate that the observed $[Au_n(MSA)_n]$ species originate from the nanoparticles collected from the gel electrophoretic separation. Provided that these signals were not the result of any artefact formation, their occurrence might indicate that the surface of the synthesised Au nanoparticles was completely or at least nearly completely (e.g., taking into account the signal at m/z 841 identified as $[Au_2MSA_3]^-$) covered by MSA molecules. These findings correlated very well with the results of the $^{197}Au^+/^{32}S^+$-ratio measurements by ICP-MS as described above. However, a final proof of the molecular composition of these nanoparticles is still pending.

In order to verify the nature of the ligand, MS/MS experiments were carried out. Exemplarily, the $[Au_4MSA_4–H]^-$ at m/z 1383 was isolated in the ion trap instrument and further fragmented as described in the experimental part. The obtained MS/MS spectrum is shown in Fig. 5. Besides the typical losses of water from the ligand MSA, fragment losses of Δm = 116 Da and Δm = 150 Da were observed. They were described for MSA complexes of Bi in similar manner [42] and can be recognised as the loss of an entire MSA molecule (Δm = 150 Da), e.g., m/z 1266.9 ($[Au_4MSA_3S]^-$) to 1117.1 ($[Au_4MSA_2S–H]^-$, and the cleavage of the S–C-bond accompanied by the loss of carbon-containing rest of MSA (Δm = 116 Da), resp. Typical examples for this type of fragmentation could be found in the mass spectrum for m/z 1382.7 to 1266.9 with a subsequent second loss of m/z = 116 Da to 1150.8 ($[Au_4MSA_2SSH]^-$). These initial data already demonstrate that ESI-MS/MS experiments hold great potential in determining and identifying the ligands covering the sought nanoparticles. Although in this study the ligand was well-known due to the synthetic route of preparing the nanoparticles the application of MS/MS techniques will most likely offer great potential in future application of mass spectrometry for the molecular identification of nanoparticles in "real world" samples, e.g., in toxicological studies.

Indeed, the initial results presented here on the ESI-MS analysis of Au nanoparticles still need improvements in terms of finding optimum conditions for the characterisation of entire nanoparticles [36]. Nevertheless, the combined approach of elemental and molecular information on these emerging elemental species might become an important tool in future nanoparticle-based research as it did already e.g., in metallomics studies [43].

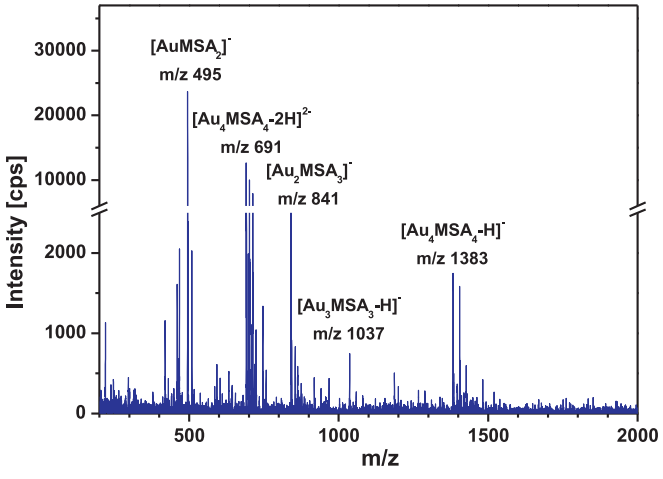

Fig. 4. Negative ESI-MS of the collected nanoparticle fraction at 7.3 min (see Fig. 3) MSA.

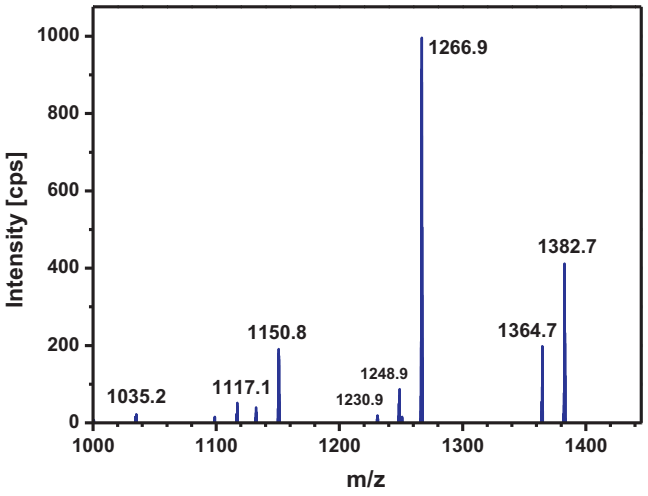

Fig. 5. MS/MS spectrum of m/z 1383 ($[Au_4MSA_4–H]^-$).

4. Conclusions

ICP-MS detection in combination with liquid chromatography and gel electrophoresis was developed for the analysis of Au nanoparticles. Although LC separations required the use of SDS as modifier in the mobile phase, it could be shown that it is a very useful tool for detailed studies on the particle formation. As demonstrated on the formation of citrate-protected Au nanoparticles the proposed LC-ICP-MS approach provided a deeper insight into the process of nanoparticle generation in solution. Besides that, the online coupling of gel electrophoresis and ICP-MS could provide essential information about the composition of nanoparticles containing a second ICP-MS detectable element. This was demonstrated on the analysis of MSA-stabilised Au nanoparticles. By simultaneous determination of the sought elements the $^{197}Au^{+}/^{32}S^{+}$-ratio was obtained for the separated nanoparticles. Here, ICP-MS based hyphenated techniques might play an important role for further characterisation of nanoparticles as ICP-MS provides a simultaneous multi-element detection and determination. However, for more comprehensive molecular information complementary mass spectrometric techniques – such as ESI-MS – are necessary in order to identify definitively nanoparticles and their chemical composition [36]. Here, a first step – as combining ICP-MS with ESI-MS data – was done, which provided useful information about the ligand's nature protecting the Au nanoparticle. In future, it can be expected that a combined approach of complementary elemental and organic mass spectrometry will give additional value to the analysis of nanoparticles in various areas related to nanoscience. An additional value can be seen in the fact that nanoparticle-bound metal ions can be easily distinguished from low-molecular complexes of the sought metals.

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