

# Silver $^{109}\text{Ag}$ Nanoparticles for Matrix-Less Mass Spectrometry of Nucleosides and Nucleic Bases

Joanna Nizioł and Tomasz Ruman

**Abstract**—The application of new  $^{109}\text{AgNPET}$  nanoparticle surface for analysis of nucleosides and nucleic bases is described along with characterization of  $^{109}\text{Ag}$  nanoparticles. The nanoparticles allow laser desorption-ionization mass spectrometry analyses of various low molecular weight (LMW) organic compounds. The new method was used for successful determination of thymidine, 5-fluorouracil, 2'-deoxycytidine, cytidine and 2'-deoxyuridine. The mass determination accuracy was in the 1-3 mDa range which confirms identity of the analyte.

**Index Terms**—Low molecular weight compounds, MALDI, mass spectrometry, matrix-assisted laser desorption-ionization, nanoparticles, SALDI.

## I. INTRODUCTION

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), developed independently by two groups in 1985 by Karas *et al.* [1], and Tanaka *et al.* in 1988 [2], belongs to the most selective, sensitive and efficient mass spectrometric methods. It offers soft ionization potential, being therefore a unique tool for the analysis of high-molecular weight compounds such as peptides, proteins, DNA/RNA, polysaccharides or polymers [3]. The most important advantages of MALDI are soft and efficient desorption-ionization of various fragile and non-volatile samples, with relatively low degree of fragmentation, high tolerance for contaminants, uncomplicated spectra as most ions are singly charged, very high detection sensitivity over a wide  $m/z$  range and remarkable detection sensitivity. Further advantages of MALDI include rapid analysis, ionization methods comparable to those used in atmospheric pressure ionization (API) mass spectrometry, such as electrospray ionization mass spectrometry (ESI-MS), and require relatively simple instrumentation.

MALDI has not been applied too regularly to detect low molecular weight (LMW) compounds, of MW <1000 Da, because common MALDI matrices are low molecular weight organics producing rather large amount of matrix-related ion peaks. One of the most important breakthroughs was described by Sunner *et al.* by introduction of the surface-assisted desorption/ionization (SALDI) technique, with graphite particles suspended in glycerol used as matrix [4]. LDI-MS analysis of small molecules involve also the use

of several types of nanoparticles (NPs) and quantum dots (QDs) applied as matrices, including Ag, Au, Pt, HgTe, ZnS,  $\text{TiO}_2$ ,  $\text{MnO}_2$ ,  $\text{Mn}_2\text{O}_3$ ,  $\text{ZrO}_2$ ,  $\text{ZrO}_2\text{-SiO}_2$ , CdSe, ZnSe, BaSrTiO, TiSiO,  $\text{Fe}_3\text{O}_4$ ,  $\text{Fe}_2\text{O}_3/\text{TiO}_2$  FePtC and diamond nanostructures [5]. Recently, our group presented first silver nanoparticle enhanced steel target (AgNPET) which was successfully used for fast and reliable analysis of LMW compounds. Not long ago, we have also presented few examples of the use of monoisotopic  $^{109}\text{AgNPET}$  method which was first application of  $^{109}\text{Ag}$  nanoparticles in mass spectrometry [6]. Present work is the continuation of  $^{109}\text{AgNPET}$  method development for analysis of rather sensitive nucleosides and nucleic bases.

## II. EXPERIMENTAL

Silver trifluoroacetate used in nanoparticle synthesis was of 99.99+ % purity (Aldrich). 2, 5-Dihydroxybenzoic acid (DHB) and polished steel target (MTP 384 type) was purchased from Bruker Daltonik GmbH (Germany). All other chemicals were purchased from Sigma-Aldrich (97-99% purity). All solvents were of HPLC quality.

The electron scanning microscope JSM-6390 LV (JEOL Co., Japan) was used. MALDI-TOF mass spectrometry experiments were performed using Bruker Autoflex Speed in reflectron time-of-flight mass spectrometer equipped with a Smart Beam-II 1 kHz laser (355 nm). Laser impulse energy was approx. 100-150  $\mu\text{J}$ , laser repetition rate - 1000 Hz, and deflection was turned on for  $m/z$  lower than 80. The first accelerating voltage was held at 19 kV, and the second ion source voltage at 16.7 kV. Reflector voltages used were 21 kV (the first) and 9.55 kV (the second). All spectra were acquired by integrating approx. 6000 shots. The data were recorded and analyzed by software provided with the Autoflex (FlexAnalysis version 3.3). Mass calibration (typically cubic calibration based on 6-9 points) was performed using internal standards (silver ions and clusters from  $^{109}\text{Ag}^+$  to  $^{109}\text{Ag}_{10}^+$ ). AgNPET LDI samples were prepared by dissolution of 1 mg of compound in 1 ml of ultra-pure water.  $^{109}\text{AgNPET}$  was prepared similarly to AgNPET [5] with exception of AgTFA which contained 99,75+% of  $^{109}\text{Ag}$  isotope (BuyIsotope, Sweden).

## III. RESULTS AND DISCUSSION

The mass spectrometry of low molecular weight compounds is currently based mainly on electrospray ionization (ESI), with time-of-flight (ToF) analyzers of various kinds. The application of ESI ionization method has many drawbacks such as (i) presence of multiply-ionized

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ions, (ii) often low clarity of spectrum, (iii) unreliable results of mixture analysis, (iv) often high degree of fragmentation of sensitive compounds. The mentioned problems could be avoided with the use of MALDI-type instrument which has the capabilities to soft-ionize analyzed samples forming almost solely single ionized ions. The traditional MALDI matrices (CHCA, DHB) are suitable rather for ionic substances, such as peptides, proteins and some polymers, characterized by the  $m/z$  value higher than 1000. Their limitations are due to (i) matrix peaks in the region of  $m/z < 1500$ , (ii) unreliable calibration, (iii) low mass determination accuracy with external calibration, (iv) low ionization potential for most of organic compounds and (v) inhomogeneous co-crystallization. What is more, acidity of the standard matrix solutions can catalyze hydrolysis of phosphorylated proteins, peptides and amino acids [7]. Although application of silver nanoparticles showed promise to overcome most of the above mentioned problems, so far presented approaches, using mostly mixtures of AgNPs with analyte, did not result in high  $m/z$  accuracy due to thickness of the analyte spot. Moreover, these methods require time- and cost-consuming isolation of AgNPs, as well as stabilizers preventing aggregation of AgNPs. The application of nanoparticles in laser desorption-ionization mass spectrometry can also be limited by the risk of nanoparticles contamination inside the MS instrument during laser ablation. As discussed above, there is a need for simple, effective method with soft-ionization potential, of wide applicability (for polar and non-polar compounds) and high sensitivity.

The mass spectrometry application of cationic silver nanoparticles allows soft ionization of most organics with even 10-point internal calibration in cubic mode which results in a very high mass accuracy of the analyte, impossible to reach with external calibration. We have shown recently results with sub-mDa mass accuracy for many LMW compounds [5], [6] which are state-of-the-art results for 1.2 m ToF-tube MALDI-type instruments.

The natural silver contains two isotopes in *ca.* 1:1 ratio which relates to two-peak pattern of monosilver-analyte adducts. The use of silver-109 isotope allows for higher sensitivity and produces much clearer multi-compound spectra.

The synthesis of  $^{109}\text{Ag}$  nanoparticles on the surface of commercially available Bruker MTP MALDI steel target was made with the DHB method as shown in our recent work [6]. The investigation of AgNPs size and shape was accomplished by various methods, including UV-VIS spectrophotometry, dynamic light scattering (DLS, [5]) and scanning electron microscopy (SEM, Fig. 1). The SEM image also roughly confirmed *ca.* 100 nm size of  $^{109}\text{Ag}$  nanoparticles as shown in Fig. 1.

The LDI mass spectrum of  $^{109}\text{AgNPET}$  in the positive reflectron mode in the  $m/z$  0 - 1500 range is very simple and clear, containing only three peaks assigned to silver ions  $^{109}\text{Ag}^+$  (calculated value - 108.9048 Da),  $^{109}\text{Ag}_2^+$  (calculated value - 217.8096 Da) and  $^{109}\text{Ag}_3^+$  (calculated value - 326.7144 Da), and few low intensity peaks of higher silver cationic species, including  $^{109}\text{Ag}_4^+$  (435.6192 Da),  $^{109}\text{Ag}_5^+$

(544.5240 Da),  $^{109}\text{Ag}_6^+$  (653.4288 Da),  $^{109}\text{Ag}_7^+$  (762.3336 Da),  $^{109}\text{Ag}_8^+$  (871.2384 Da),  $^{109}\text{Ag}_9^+$  (980.1432 Da),  $^{109}\text{Ag}_{10}^+$  (1089.048 Da) and higher-mass species even up to  $^{109}\text{Ag}_{30}^+$  [6]. The peaks of monoisotopic ions show very simple isotopic patterns, with basically no  $^{107}\text{Ag}$  isotope (lower than 0.25%). In order to test the applicability of  $^{109}\text{AgNPET}$  to ionize nucleosides and nucleic bases, few representative compounds were selected. Each of the compounds was tested using stock solution of 1 mg/ml concentration. Thymidine MS spectrum obtained with the use of  $^{109}\text{AgNPET}$  (Fig. 2; upper panel) shows two characteristic peaks, assigned to thymidine-silver adduct at  $m/z$  350.9939 and also contaminant - [thymine+ $^{109}\text{Ag}$ ] $^+$  adduct at  $m/z$  234.9493. The signal-to-noise (S/N) ratio for [thymidine+ $^{109}\text{Ag}$ ] $^+$  peak is 1331.

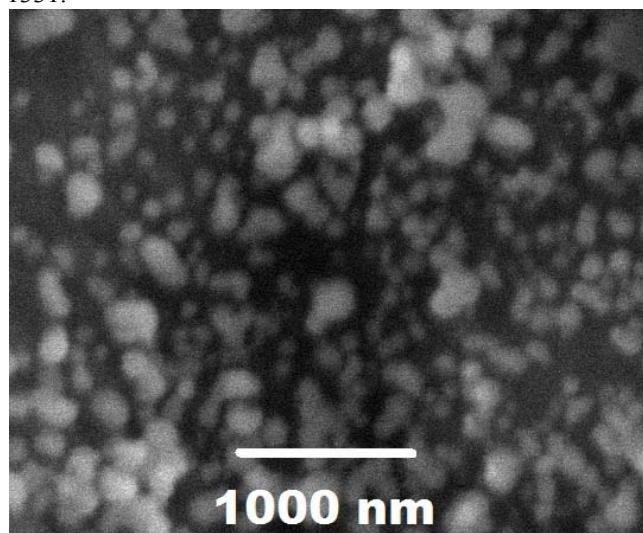


Fig. 1. SEM image of  $^{109}\text{AgNPET}$  surface at 15,000 magnification.

Noteworthy is that thymidine-silver adduct  $m/z$  value could be determined with high accuracy, defined as the difference between calculated and experimental mass, and was found to be 0.0012, relating to *ca.* 3 ppm. Another important compound - anticancer drug 5-fluorouracil was investigated with the same method (Fig. 2, bottom). The silver-109 adduct was found to be at  $m/z$  238.9258 with only 0.003 difference.

The MS spectra of cytidine, 2'-deoxycytidine and 2'-deoxyuridine with the use of  $^{109}\text{AgNPET}$  are presented in Fig. 3. All of mentioned compounds form silver-109 adducts of [cytidine+ $^{109}\text{Ag}$ ] $^+$  (351.9879 Da; S/N ratio 824), [2'-deoxycytidine+ $^{109}\text{Ag}$ ] $^+$  (335.9945 Da; S/N ratio 247) and [2'-deoxyuridine+ $^{109}\text{Ag}$ ] $^+$  (336.9807 Da; S/N ratio 987) type which are visible as single peaks. The calculated  $m/z$  differences are 0.003, 0.001 and 0.001 respectively. Spectra (Fig. 3) contain also peaks of contaminants - nucleic bases - cytosine and uracil.

#### IV. CONCLUSION

Monoisotopic silver-109 nanoparticle-enhanced targets ( $^{109}\text{AgNPET}$ ) were used for analysis of low molecular weight (LMW) compounds. Due to sub-micrometer thickness of active surface and possibility of precise up to 10 point calibration, using  $^{109}\text{Ag}^+$  to  $^{109}\text{Ag}_{10}^+$  ion peaks, each present a

single, high intensity line. The laser desorption-ionization (LDI) mass spectra with  $^{109}\text{AgNPET}$  were suitable for analysis of sensitive compounds such as nucleosides - thymidine, 2'-deoxycytidine, cytidine and 2'-deoxyuridine,

and also unnatural nucleic base - 5-fluorouracil, and allowed very high mass determination accuracy.

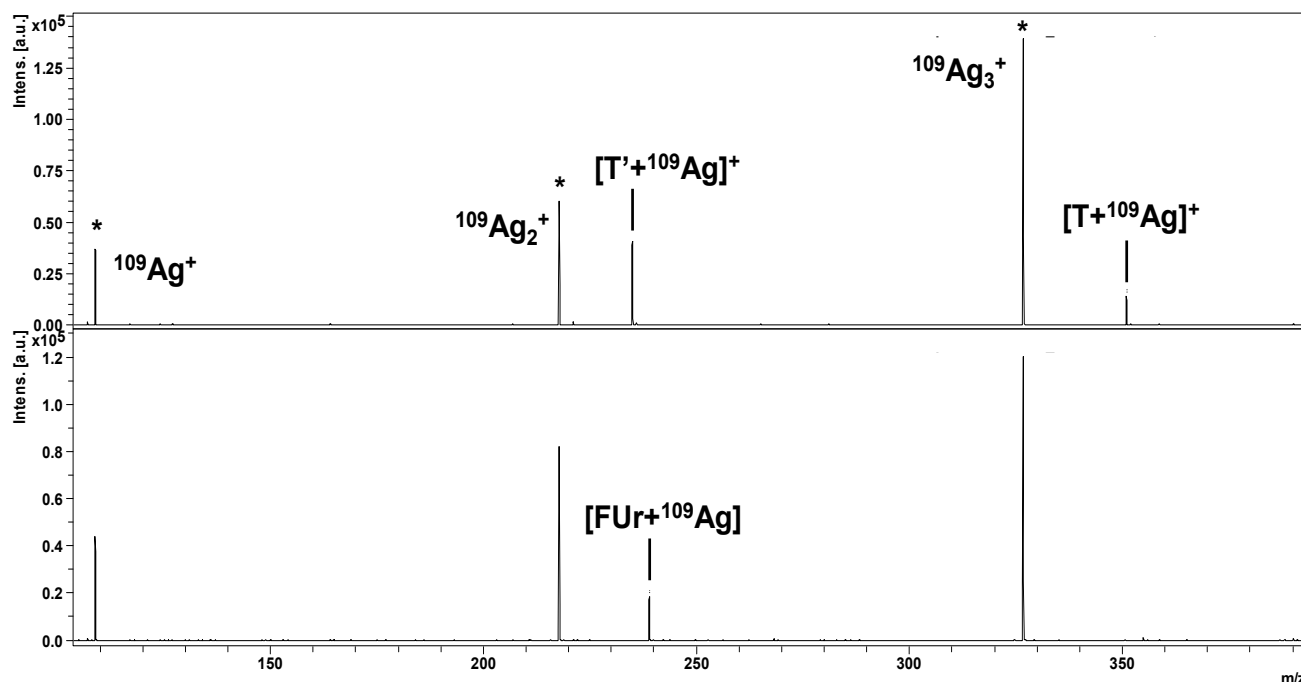


Fig. 2.  $^{109}\text{AgNPET}$  MS spectra of thymidine (top panel) and 5-fluorouracil (bottom panel). T' - thymine; FUR - 5-fluorouracil.

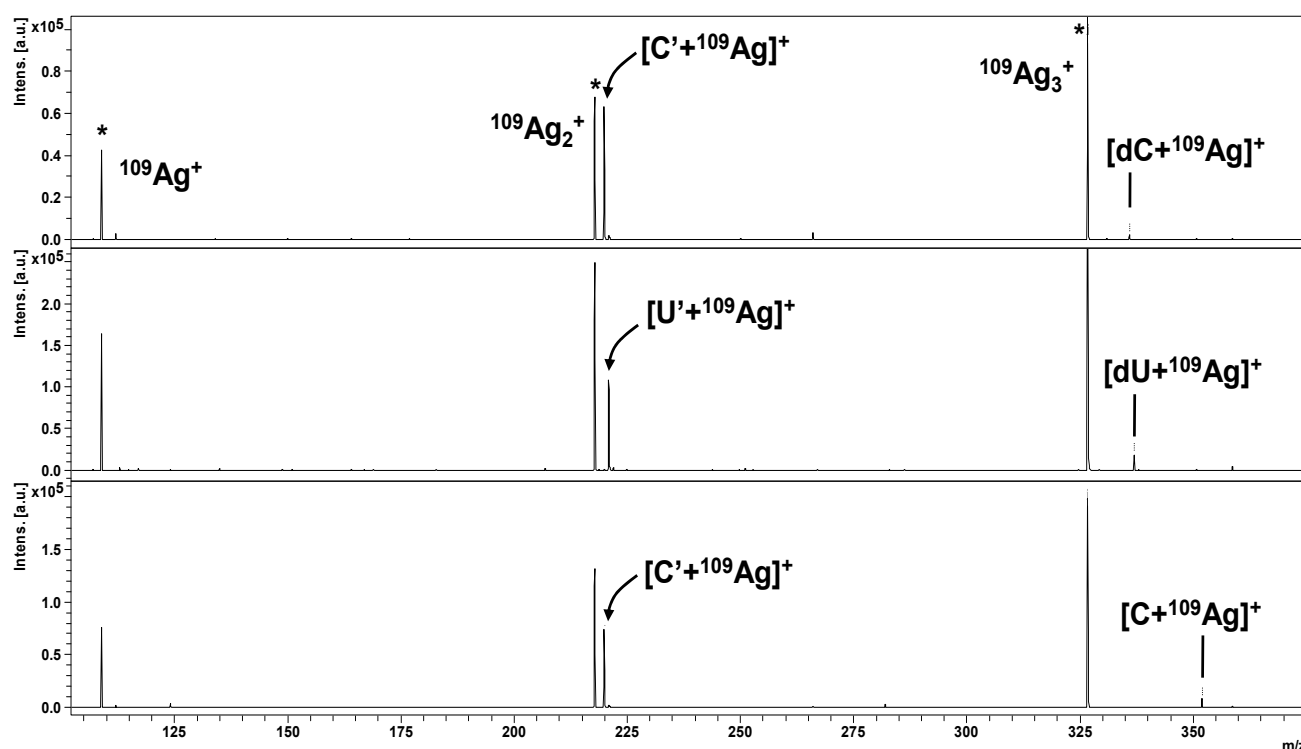


Fig. 3.  $^{109}\text{AgNPET}$  MS spectra of 2'-deoxycytidine (top panel), 2'-deoxyuridine (middle panel) and cytidine (bottom panel), C' - cytosine; U' - uracil.

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desorption/ionization mass spectrometry.

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