Contributions to quantitative analysis of nanomaterials: Microscopic investigations of collected aerosols of nanoparticle solutions

Tobias J. Fiedler, Jan-Hendrik Arndt, Jan O. Orlandini von Niessen, Nicolas H. Bings

Johannes Gutenberg-University Mainz, Institute for Inorganic and Analytical Chemistry, Laboratory for Inorganic Trace Analysis and Plasma Spectrochemistry
Duesbergweg 10-14, 55128 Mainz, Germany, tobias.fiedler@uni-mainz.de

Introduction

For the analytical characterization of nanoscale particles (NP), used for many technical, pharmaceutical and diagnostic applications, it is important to determine, in which specific biological tissue NPs predominantly accumulate. It is scientifically accepted, that nanoparticles pass the mucosal barriers through different pathways and that the mucosal cells are able to take up solution and particles of up to 10 µm [1-3]. Here the significance of these pathways is still unknown. A common method to characterize nanoscale materials is transmission electron microscopy (TEM). Due to the low concentration of nanoparticles in tissue the use of very sensitive microscopic methods like two-photon-laser-scanning microscopy is necessary to determine NPs and thus to identify health effects of such particles. But even these techniques are often not sensitive enough and provide only qualitative information. The quantitative analysis of NPs without digestion is still difficult, because of the size, stability and handling of the nanoparticles.

Inductively coupled plasma mass spectrometry (ICP-MS) is a powerful tool for quantitative trace analysis of most metals. In the case of Au nanoparticles in low concentrations, ICP-MS can be used for the determination of the concentration as well as the size distribution via coupling with separation techniques. Quantitation of NPs is easily possible, if suitable nanoparticle solutions are available since aqueous standards can not be used [4]. Nanoparticle solutions with known concentrations are hard to obtain. It is still hardly possible to determinate the concentration of nanoparticles in a solution using standard solutions for calibration. Nanoparticle and standard solutions of equal metal concentration achieve different ICP-MS sensitivities, thus mathematical correction using a constant sensitivity factor becomes necessary. As sample introduction is crucible in ICP-MS, the process from sampling to ionization has to be investigated and optimized for reliable quantification of NPs using aqueous standards. The first aim therefore has to be to determine if current sample introduction systems alter size and morphology of the used nanoparticles.

Various nebulizers can be used for sample introduction into the ICP. Due to a broad droplet size distribution and limited nebulizing efficiency, usually more than 90 % of the used solution is basically wasted. A recently developed sample introduction system is the *"drop-on-demand"* (DOD) aerosol generator. This system is based on a commercial inkjet cartridge which is capable of reproducibly producing small droplets in the *pL*-range [5].

Results

Each sample was measured with TEM at different magnifications (21.000x (left image) and 55.000x (right image)). Fig. 1 shows nanoparticles directly pipetted onto a TEM grid from solution as a reference. It is recognizable that the size of the nanoparticles is about 16 nm. Fig. 2 and Fig. 3 display nanoparticles, nebulized with commercial nebulizers (Fig. 2 MicroMist, Fig. 3 EnyaMist). With the DOD system it is also possible to dose nanoparticle solutions. No differences in shape and size are visible between solutions, which where either collected from the DOD aerosol and pipetted (5 uL) onto the TEM grid, or were directly transferred onto the grid trough the DOD (Fig. 4, Fig. 5). As shown in Fig. 6 there is no significant change in size of the nanoparticles, whether they are nebulized with a commercial nebulizer or the new DOD system, as determined from the images. Also, no significant change in shape is visible for the nanoparticles, nebulized with all three investigated sample introduction systems.

References

[1] Ermak, T.H., Dougherty, E.P., Bhagat, H.R., Kabok, Z., Pappo, J.; *Cell Tissue Res.*, 1995, **279**, 433.
[2] Neutra, M.R, Pringault, E., Kraehenbuhl, J.P.; *Annu. Rev. Immunol.*, 1996, **14**, 275.
[3] Beier, R., Gebert, A.; *Am. J. Physiol. Gastrointest Liver Physiol.*, 1998, **275**, 130.
[4] Vogel, N., Hauser, C. P., Schuller, K. Landfester, K. Weiss, C. K.; *Macromol. Chem. Phys.*, 2010, **211**, 1355.
[5] Orlandini v. Niessen, J. O., Schaper, J. N., Petersen, J. H. Bings, N. H.; *J. Anal. At. Spectrom.*, 2011, **26**, 1781.

Experimental settings

Since nanoparticle solutions might often be valuable and biological sample volumes are often very limited, sample consumption should be kept at absolute minimum. In this study current commercial low-flow nebulizers e.g. concentric and parallel path nebulizers are compared to the DOD system regarding a potential identity change and formation of agglomerates of NPs due to nebulization. All measurements were done using the same nanoparticle solution, characterized by small angel light scattering (SAXS): 0.25 mM solution of Au nanoparticles, r = 7.12 nm, PI = 1.2. All nebulizers were used under standard operating conditions. As reference the original solution was pipetted directly onto a TEM grid (Carbon Film on 300 square Mesh Cu grids). Increase of the DOD system droplets were also collected in a vial and pipetted onto grids. The solution were nebulized using the different systems and aerosol was collected directly onto grids. The TEM grids were dried in vacuum before microscopic investigation of the dried residuals.

TEM images of nanoparticles after different dosing processes: Comparison of distribution

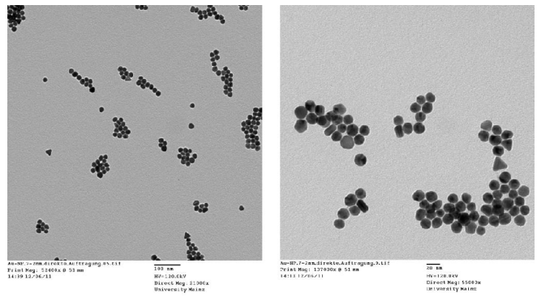

Fig. 1: Direct pipetting of 5 µL nanoparticle solution onto a TEM grid(reference)

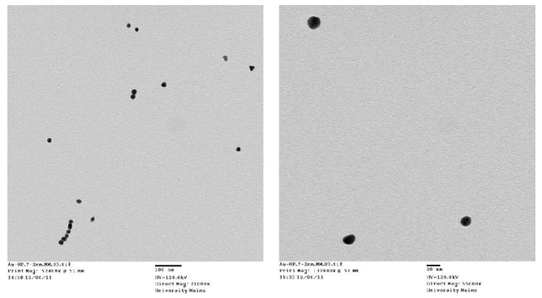

Fig. 2: TEM grid were held directly into spray of the MicroMist

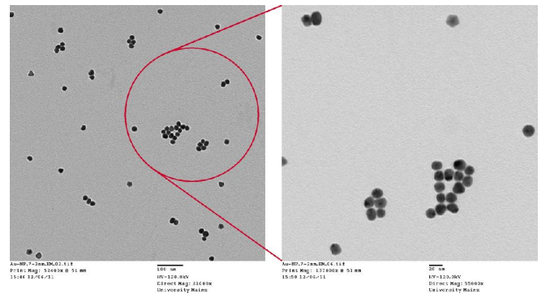

Fig. 3: TEM grids were held directly into spray of the EnyaMist

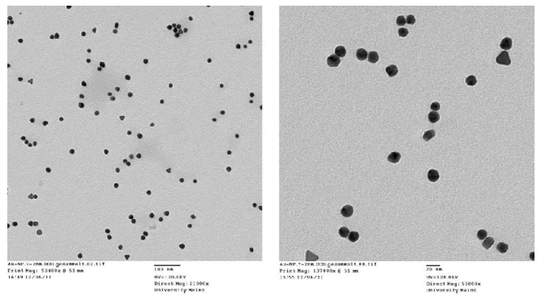

Fig. 4: Pipetting of 5 µL collected nanoparticle solution from the DOD onto a TEM grid

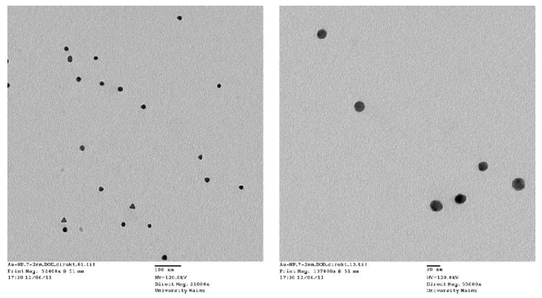

Fig. 5: Direct dosing of nanoparticle solution with the DOD system onto a TEM grid

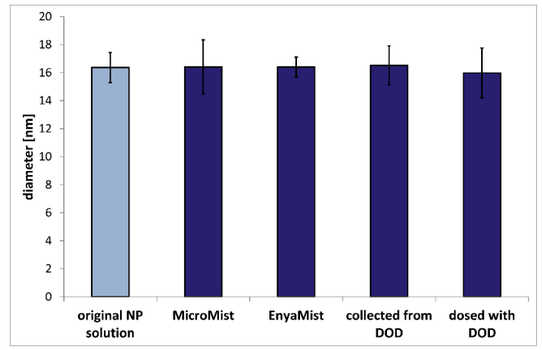

Fig. 6: Comparison of the size of nanoparticles after nebulization using different sampling systems (magnification 55.000x)

Conclusion

- nanoparticles in the solution investigated with TEM showed an about 2 nm larger diameter compared to SAXS measurements
- commercial nebulizers are applicable for aqueous nanoparticle solutions
- DOD system can be applied to dosing nanoparticle solutions
- no difference in the size and shape of nanoparticles after nebulization
- bigger droplets lead to agglomeration of NPs

➠ influence of nebulization process on the size and morphology of the NP was found to be not significant

Outlook

- comparison of sensitivities of standard versus nanoparticle solutions for calibration
- influence of different solvents on the sensitivity correction factor for calibration
- size-exclusion chromatography for separation of nanoparticles of different sizes

Acknowledgment

The authors would like to thank the BAM (Bundesanstalt für Materialforschung und -prüfung) for providing nanoparticle solution as well as the GDCh (Gesellschaft Deutscher Chemiker) for financial support.