The analytical toolbox for detection and characterization of silver nanoparticles in rat tissues from an \textit{in-vivo} toxicological study

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Detection/characterisation/ADME in animals
1. NanoTest Toxicity of engineered nanoparticles (Ag, Se)
2. NanoLyse Analysis and detection of nanoparticles in the food matrix (Ag, SiO$_2$, gelatine, fullerene)
3. NanoPack Nano-reinforced food contact materials

Toxicology
Pathology

Food technology
1. Electrospun nano-carries of nutrients
2. Gelatine nano-carriers for PUFAs
Silver nanoparticles in food and food contact materials

http://www.nanotechproject.org/
The nanoparticle separation and detection platform

Asymmetric flow field flow fractionation

Particle separation according to their size (small NPs elute first)

Optical detection (multi angle and dynamic light scattering, UV and fluorescence)

Particle detection (fractogram)

Size determination (root mean square, hydrodynamic and geometric radius)

Inductively coupled plasma mass spectrometry (ICP-MS)

Elemental detection for identification of particles

Quantification

[Graphs and data visualization showing m/z values for different elements like Zr, Ce, Ba]
Influence of AF$_4$ cross flow settings (mL/min) on retention volume Ag NPs from NanoLyse (www.nanolyse.eu)

Löschner et al., J. Chrom. A (In press)
Determination of nanoparticle size distribution

Calibration with size standards (polystyrene nanospheres) in 0.05% SDS, N=2-3

Löschner et al., J. Chrom. A (In press)
Determination of silver nanoparticle size distribution @ 1.0 mL/min cross flow

Calibration with size standards (polymer nanoparticles)

MALS not possible for sizing because Ag is an absorbing Nanoparticle and DLS is insensitive

Löschner et al., J. Chrom. A (In press)
Determination of nanoparticle size distribution

Fraction collection for transmission electron microscopy (TEM)

not quantitative (yet)

Löschner et al., J. Chrom. A. (In press)
Exposure to and detection of nanoparticles in biological material. NanoTest project

28 days dosage via sonde in GI tract of: AgNPs (14 nm o.d.), or AgAc (dissolved silver; Ag⁺)

Distribution in organs
Determination of Ag by ICPMS in tissue following ashing with HNO₃/HCl

Is silver still in particle form?
Distribution within cells?
Have the particles agglomerated?
Imaging by TEM/EDX of thin tissue slices (50 – 100 nm).

Distribution within organs
Silver staining (as in photography) of tissue slices followed by light microscopy

http://ratguide.com/
Stabilised silver nanoparticles analysed by batch-mode DLS

- Dynamic light scattering (DLS) and transmission electron microscopy (TEM)
- 14 nm
- Approx. 10% of Ag as Ag⁺ or clusters (12.5 kDa filter)
- Long-term stable (150 d)

AgNP + PVP

Volume (%)
Organ distribution of silver – silver nanoparticles vs. silver acetate

The diagram illustrates the concentration of silver (Ag) in various organs (small intestine, stomach, liver, kidney, lungs, muscle, brain, plasma) after exposure to silver nanoparticles vs. silver acetate. The concentration is measured in ng/g wet weight. The data suggests a significantly higher concentration of Ag in the small intestine for both nanoparticles and acetate, with the nanoparticles showing a slightly higher range compared to acetate.
Organ distribution of silver – silver nanoparticles vs. silver acetate

[Bar chart showing the concentration of Ag (ng/g wet weight) in different organs: small intestine, stomach, liver, kidney, lungs, muscle, brain, plasma. The chart compares Ag nanoparticles and Ag acetate.]

Ag nanoparticles
Ag acetate
Organ distribution of silver – silver nanoparticles vs. silver acetate

<table>
<thead>
<tr>
<th>Organ</th>
<th>Ag nanoparticles</th>
<th>Ag acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>small intestine</td>
<td>20,000</td>
<td>30,000</td>
</tr>
<tr>
<td>stomach</td>
<td>5,000</td>
<td>12,6 mg/kg bw/day</td>
</tr>
<tr>
<td>liver</td>
<td>1,000</td>
<td>9.2 mg/kg bw/day</td>
</tr>
<tr>
<td>kidney</td>
<td>45,000</td>
<td>*</td>
</tr>
<tr>
<td>lungs</td>
<td>2,000</td>
<td>*</td>
</tr>
<tr>
<td>muscle</td>
<td>1,500</td>
<td>*</td>
</tr>
<tr>
<td>brain</td>
<td>1,000</td>
<td>*</td>
</tr>
<tr>
<td>plasma</td>
<td>500</td>
<td>*</td>
</tr>
</tbody>
</table>

*p < 0.05
Light microscopy / autometallographic staining

Silver nanoparticle exposed rat: ileum

intestinal villi

silver

500µm

50µm
Transmission electron microscopy (TEM)

Silver nanoparticle exposed rat: ileum

intestinal villus  lysosome containing particles
Transmission electron microscopy (TEM)

Silver nanoparticle exposed rat: ileum

intestinal villus

particles in the basal lamina
Transmission electron microscopy (TEM)

Silver acetate exposed rat: ileum

lysosome containing particles

particles in the basal lamina
Question: What can we learn about the chemical composition of AgNPs in rat intestinal cells?

TEM+ energy dispersive X-ray spectroscopy (EDX)
Are silver NPs more harmful to rats than dissolved silver?

Control
AgNPs (9 mg Ag/kg bw/day)
AgAc (9 mg Ag/kg bw/day)

Bodymass decrease

Relative mass of thymus

Plasma alkaline phosphatase

Plasma urea

NOAEL:
AgNPs: = 9 mg Ag/kg bw/day
AgAc: < 9 mg Ag/kg bw/day

Reference: N. Hadrup et al, accepteret til: "Archives of Toxicology" september 2011
Urine metabolome of rats following AgNP dosage:
Female rats group separately
from their controls and from males

Female vehicle
Female high NP
Male vehicle
Male high NP

Females, 9 mg/kg b.w. as AgNPs
Urine metabolome of rats: Female rats group separately from their controls, but not by dosage level nor by AgNPs vs. AgAc.
**Metabolomics by LC-Q-TOF-MS:**
Excretion of uric acid and allantoin were enhanced in female rats’ urine

**Biochemical interpretation:**
Uric acid and allantoin may be increased due to ROS formation caused by exposure to AgNPs and AgAc

Resumé

• A large and varied box of tools and multidiciplinary collaboration is necessary in nanotox studies

• AgNPs or AgAc are distributed equally in the rat

• Silver, irrespective of the dosage form, exists as nanoparticles in intestinal cells

• Our research indicates that the AgNPs are (partially) dissolved and re-deposit as NPs in the cells

• Toxicological experiments with rats indicate, that AgNPs are equally or less toxic than AgAc for the investigated end-points

• 1000 $-$-question: Is it safe to recommend the use of AgNPs in contact with food?
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Announcement:

FFF hands-on training workshop

When: 
April 17-19, 2013

Where: 
UVIE in Vienna or DTU in Copenhagen

How: 
Sign up and follow instructions on www.nanolyse.eu