In vitro human digestion test to monitor the dissolution of silver nanoparticles

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Increasing industrial production and use of commercial goods containing silver nanomaterials likely human oral exposure

Risk assessment model in conditions simulating human ingestion

Quantification of bioaccessibility/availability

products of biotransformation
- dissolved ions
- aggregates/agglomerates
- nanosized particles
**The model**

**In vitro human digestion model:**
It simulates the human digestion in the oral, gastric and intestinal compartments with salt and protein composition, pH differences and transit times alike the *in vivo* digestion.

Dynamic process

- 5 minutes
  - pH 6.8
- 120 minutes
  - pH 2.5
- 120 minutes
  - pH 6.5

=> **useful** analytical tool to measure bioaccessibility and bioavailability of drugs or food contaminants

Our aim

- **Size**
- **Dissolution**
- **Agglomeration**

surrounding environment
dependent properties
Our approach

- **NM300k** (*klein et al, 2011*): reference nanoparticles in many European projects in nanoregulatory context

- **Pre-Standard Operational Procedures (SOPs)**
  - material preparation
  - probe sonication
  - TEM grids
  - matrix juices
  - instrument use

- **Multi-technique approach**: to gain complementary information
  - TEM
  - DLS
  - UV-Vis
  - UF/ICP-AES
NM300k in dispersion medium

**Panel A:** Image showing dispersion medium with particles, with a measurement of particle size as $d: 15 \pm 5 \text{ nm}$.

**Panel B1:** Histogram showing major axis distribution with $d: 17 \pm 5.5 \text{ nm}$.

**Panel B2:** Histogram showing minor axis distribution with $d: 14 \pm 4.5 \text{ nm}$.

**Panel C:** Size distribution histogram with $H_0: 40 \pm 3 \text{ nm}$.

**Panel D:** Zeta potential distribution with $\zeta = -24 \pm 5 \text{ mV}$.

**Panel E:** Absorption spectrum with $\lambda_{abs} = 412 \text{ nm}$.
NPs almost show the primary size and tend to form agglomerated structures
AM300k in stomach

- NPs strongly reduce the mean diameter, as also evidenced by the lack of plasmonic peak, and dissolve

- Big agglomerates embedded in organic matrix
- Absence of UV signal for inorganic nanoparticles
- 2% of free ions available for intestinal adsorption
- Presence of big aggregates and few nanosilver salts of different nature
NM300k

Mouth

Stomach

Small intestine

19% free ions
81% matrix-bond ions

2% free ions
98% matrix-bond ions

90-100% dissolution

Pristine NM300K
Digestive proteins
Biotransformed NPs or nanosized salts
Ag⁺ ions
Organic Matrix
Inorganic matrix
Bile

Bove et al, submitted
Evaluation of test predictability (1)

This *in vitro* dissolution test may be relevant for the risk assessment of AgNPs:

1. majority of the initial NPs is dissolved in ions $\Rightarrow$ NP exposure levels are similar to those of corresponding saline form

2. ions appear to be mostly bound to the matrix $\Rightarrow$ they may follow the same excretion pathway of the saline silver
# Evaluation of test predictability (2)

**Read-across based hypothesis**

<table>
<thead>
<tr>
<th>Ag excreted (<em>in vitro</em>)</th>
<th>Ag excreted (<em>in vivo</em>)</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>98%</td>
<td>60%</td>
<td>60-99%</td>
</tr>
<tr>
<td></td>
<td>(faeces)</td>
<td>(Bergin <em>et al</em>, 2016)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ag absorbed (<em>in vitro</em>)</th>
<th>Ag absorbed (<em>in vivo</em>)</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>0.06%</td>
<td>0.4-10%</td>
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<tr>
<td></td>
<td>(blood)</td>
<td>(Loeschner <em>et al</em>, 2011; van der Zande <em>et al</em>, 2012)</td>
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<td></td>
<td>0.02%</td>
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<td>(urines)</td>
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<td></td>
<td>1.92%</td>
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<tr>
<td></td>
<td>(tissues)</td>
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*Bove et al, submitted*
Conclusions

The dissolution test may be a valid analytical tool for nanoregulation:

=> It allows to quantify the silver nanoparticles biotransformation, through read-across of saline form