The role of single particle ICP-MS with microsecond time resolution in a multi-technique approach for unveiling the transformations of ingested metal-based nanoparticles

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Peculiar fate of ingested nanomaterials

Nanoparticles entering the human body via the oral route are subjected to **conditions that are very different from those encountered via other exposure routes**

Physicochemical properties of any ingested particulate material will be affected by:

- extreme **pH** and **ionic strength** shifts encountered during gut transit

Low pH

Stomach

pH increase

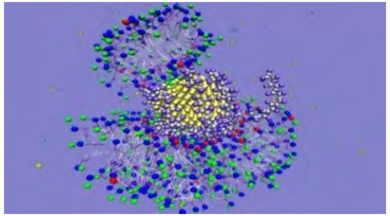
Small intestine

Ionic strenght

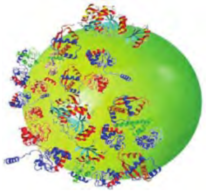
Stomach & intestine

- the **co-ingested food material**

Interaction between particles and food components



- the **enzymes and bile acids secreted** within the gut



Changes promoted by interaction with the GI environment

The extremely **low pH** of the stomach may promote:

- **oxidation/dissolution** of nanoparticles constituted by soluble metals/metal oxides with **release of constituent ions**

The **high ionic strength** in the stomach and intestine critically affect nanomaterial properties, in many cases leading to:

- **agglomeration** in the gastric phase
- **deagglomeration** in the intestinal phase

Important factors affecting **degradation/dissolution** and **agglomeration/deagglomeration** are expected to include **physical forces, temperature, pH, presence of enzymes, salts and bile, interactions with food components**

[Peters et al. 2012, ACS Nano 6:2441; Walczak et al. 2013, Nanotoxicol 7:1198; Sieg et al. 2017, Langmuir 33:10726; DeLoid et al. 2017b, Part Fibre Toxicol 14:40]

In vitro digestion models to assess the fate of ingested NMs

Changes in:
size-related properties
shape
surface characteristics

May affect the:
intestinal uptake of the particles
kinetic behaviour of the particles

Overall, interactions of nanoparticles with the gastrointestinal environment critically affect their **biological and toxicological properties**

Acellular *in vitro* methods simulating human digestion and mimicking physiological conditions *in vivo* as tools to assess the modifications/dissolution of nanomaterials

There is a **lack of validation** and **standardisation** of *in vitro* digestion models for nanomaterials

EFSA GUIDANCE – *in vitro* simulated GI digestion

Time-dependent transformations of real world particulate materials under conditions representative of the gastrointestinal tract have been studied by robust *in vitro* digestion methods **with standardised protocols** in the:

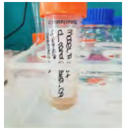
- Nanomaterials that **quickly dissolve/degrade** in the gastrointestinal tract do not give rise to nano-specific concerns and **standard risk assessment can be followed**

- If nanoparticles persist as such after gastrointestinal digestion **they may be absorbed in the gut** and **nanospecific risk assessment is required**

In vitro simulated GI digestion

Assessment of the degradation rate of nanomaterials in conditions representative of the human gastrointestinal tract is considered the **key first step**

An *in vitro* digestion method suitable for food under **fed conditions** has been described by Minekus *et al.* (2014)



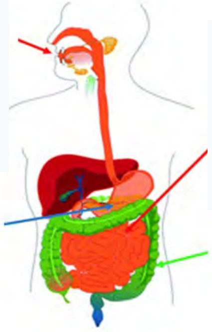
FED
Model food + nanoparticles

FASTED
Dispersion of nanoparticles

Less representative of the use of nanomaterials in food products (but may be better for food supplements): **low pH conditions** *in the stomach may promote the dissolution of most metals and metal oxides*

Oral phase
Salivary amylase, 2 min, pH 7

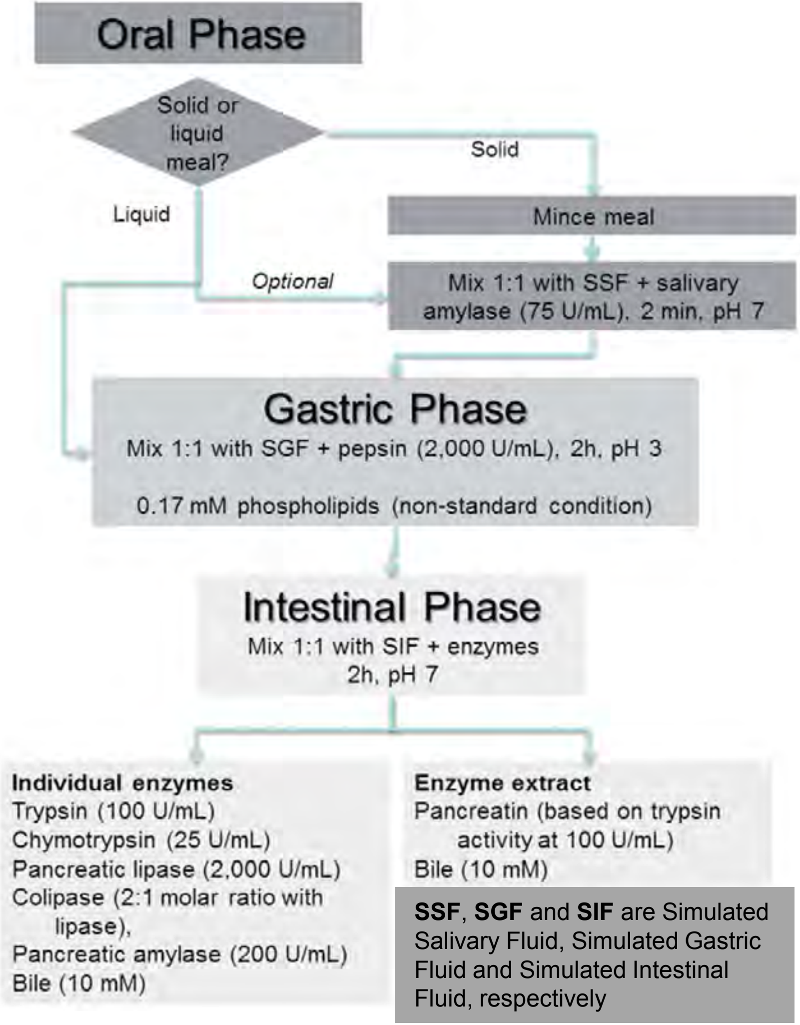
Gastric phase
Gastric fluid + pepsin, 2 h, pH 3



Intestinal phase
Intestinal fluid and enzymes, 2h, pH 7

Representative of the physiological state

[EFSA NanoGuidance 2018; Minekus et al. 2014, Food Funct 5:1113]



Mouth

Stomach

Small intestine

Minekus et al. (2014)
Food Funct. 5:1113

Sampling

Pristine material
+
Material in the food matrix
(before digestion)

Material after intestinal phase
Time points:
5, 15, 30 and 60 min

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EFSA GUIDANCE – *in vitro* simulated GI digestion

A nanomaterial is considered to **degrade quickly** (i.e. have a high degradation rate) if the degradation rate profile in the intestinal phase shows a **clear decrease in the presence of particles over time** (**no plateau**), and that **12% or less of the material** (**mass-based**) – compared with the particulate concentration at the beginning of the *in vitro* digestion – is present as particles **after 30 min of intestinal digestion**

As a sub-argument, it is also assumed that even if a fraction of such quickly degrading materials is absorbed as particles, it **is expected that further degradation will occur under e.g. lysosomal conditions** and that they are unlikely to remain as particles for a long time

The nanomaterials studied:

Ten different food-grade particulate materials belonging to four chemical classes were studied:

Synthetic amorphous silica	4 samples of the food additive (E551): 2 representative of food-related applications from the JRC repository, 2 from commercial suppliers
Titanium dioxide	2 different samples of the food color (E171) from commercial suppliers
Iron oxides/hydroxides	2 samples of the food color (E172) from commercial suppliers: one Yellow iron oxide (FeO(OH)) and one Red iron oxide (Fe_2O_3)
Zinc oxide	2 samples: one used as nutrient source for human consumption, the other one for animal consumption

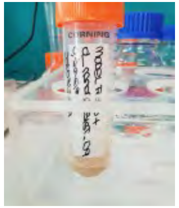
The experimental procedure

Cereal based model food

Composition of model food:

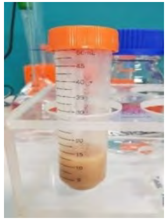
- ☐ Fats: 5%
- ☐ Carbohydrates : 11%
- ☐ Fiber: 3%
- ☐ Proteins: 11%
- ☐ Salt: 2%

The model food was homogenized and a part was stored for further studies



2'
Salivary SF

2h
Gastric SF



30'
Intestinal SF

Prior in vitro digestion, the model food was spiked with a nanoparticles dispersion and **incubated for at least one hour at room temperature**

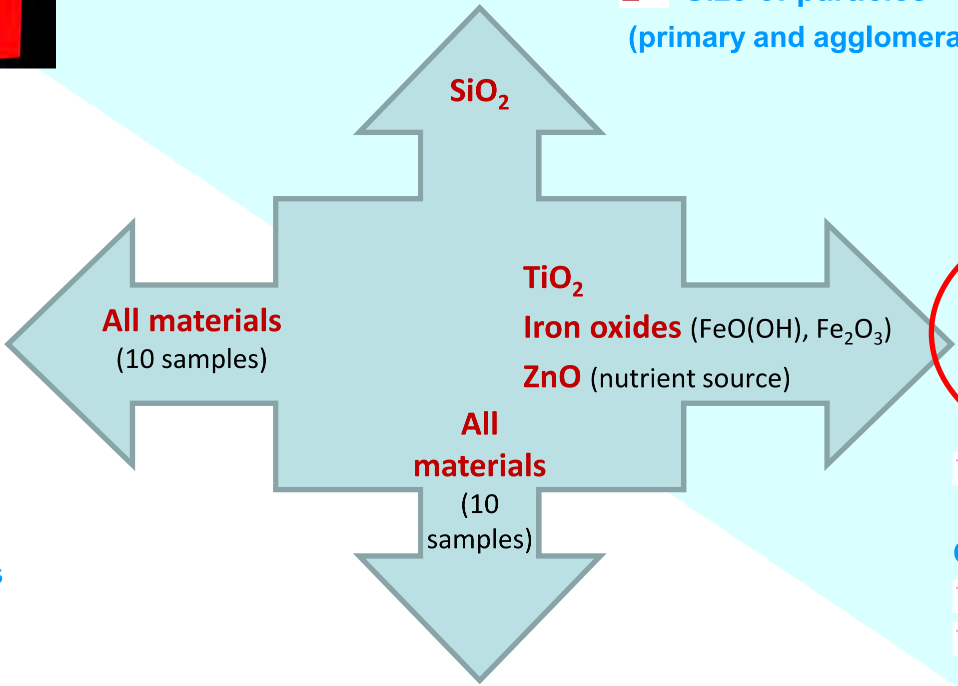
Physicochemical characterization: multi-method approach

Physicochemical characterization of the particles in pristine conditions

Asymmetric flow field flow fractionation-ICP-MS
(AF4-ICP-MS)

- Size of particles (primary and agglomerates)

MS-based techniques



Transmission electron microscopy
(TEM)

- Size of primary particles
- Shape of primary particles
- Imaging of agglomerates

Single particle ICP-MS
(spICP-MS)

- Size of particles (primary and agglomerates)

Quantitative data:

- mass concentration
- number-based distributions

Centrifugal liquid sedimentation (CLS)
Dinamic light scattering (DLS)

- Agglomeration behaviour of the pristine material (different pH)

Physicochemical characterization of pristine materials: the importance of sp IC-MS

Titanium dioxide (TiO_2)

Similar results obtained by spICP-MS : **small agglomerates** in addition to primaries (visible by **higher frequencies at larger diameters)**

Physicochemical characterization: multi-method approach

2

Characterization

After ingestion: Fed and fasted conditions

After lysosomal processing

Asymmetric flow field flow fractionation-ICP-MS (AF4-ICP-MS)

- Size of particles (primary and agglomerates)

MS-based techniques

SiO_2

All materials (10 samples)

TiO_2
Iron oxides (FeO(OH), Fe_2O_3)
ZnO (nutrient source)

All materials (10 samples)

Transmission electron microscopy (TEM)

- Size of primary particles
- Shape of primary particles
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Agglomeration behaviour of the pristine material (different pH)

Preliminary results post gastointestinal *in vitro* digestion- sp ICP-MS

Similar to the distribution obtained for the characterization of
pristine TiO2 by sp ICP-MS

Conclusions

- **Nanoparticles** entering the human body via the **oral route** are subjected to conditions that are very different from those encountered via other exposure routes
- Physicochemical properties of ingested particulate material are affected by **pH** and **ionic strength shifts, co-ingested food material, enzymes** secreted within the gut. This environment critically affect the **biological** and **toxicological** properties of the ingested nanoparticles
- Time-dependent transformations of **real world particulate** materials applying the in vitro acellular methods were studied according to the assessment scheme of the EFSA Guidance
- **Ten** different **food-grade particulate materials** belonging to **four chemical classes** were studied and the focus was on checking **robustness of protocols** for possible standardisation
- A state-of-the-art **multi-technique** approach was used for the **physicochemical characterization** of the materials before and after the tests
- **Single particle ICP-MS** as an **essential technique** in order to characterize nanomaterials after the simulated *in vitro* GI digestion

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THANK YOU FOR YOUR ATTENTION !