Nanoparticles in food – Analytical methods for detection and characterisation

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5 Years Nanotechnology Dialogue
The frame

- FP7-Project (Theme 2: Food, Agriculture and Fisheries, and Biotechnologies),
- Collaborative project, Grant agreement n° 245162, 3 M€ EC contribution
- Duration 01/2010 - 09/2013
- 10 partners
- Coordinator: RIKILT
Focus

- **Food safety**
  - presence (yes/no)
  - identity (chem. composition, size)
  - concentration (mass, number)
Objectives

- Reference materials for ENP in food
- Validated methods for nanoparticles (NP) in food matrix
  - Different scenarios: migration, addition, nano-fraction of additives, environmental contamination
  - Different NP classes
    - metals (Ag)
    - oxides (SiO$_2$)
    - carbon NP (C$_{60}$)
    - organic NP (nano-carrier systems, encapsulates)
Dual Concept

Imaging (in matrix), screening (WP2)
- detection (yes/no)
- rapid, high throughput, cost efficient, robust

Confirmatory methods
(WP3 inorganic NP, WP4 org. NP)
- unambiguous identification
- quantification
WP1: Reference materials

- Supply of characterised ENP suspensions
  - Ag, SiO$_2$, C$_{60}$, x-linked gelatine NP
- Synthesis of labelled analogues
  - Au-labelled Ag and Ge-labelled SiO$_2$ produced
  - use as internal/recovery standards

- Method validation approach for ENP in food
  - in press, comments via website
  - goal: harmonised validation guidelines for NP analysis (analogous to 2002/657/EC)
WP1: Reference materials in matrix

Ag in chicken meat
  • Transfer from food contact material

SiO$_2$ in tomato soup
  • Use of addititves (E551)

C$_{60}$ in edible oil
  • Enrichment from environmental contamination

Organic NP in beverage
  • Addition of encapsulated ingredients (vitamins, colourants, antioxidants)
WP2: Imaging

Electron microscopy

- SEM and TEM (+ EDX) methods for Ag NP (meat) + SiO_2 NP (soup)

Sample prep

- Comparison of different methods: blotting, ultra-centrifugation, air/freeze/chemical drying, resin embedding

Image analysis

- Automated image analysis required
- object-based software prototype
WP2: Screening

SPR Biosensor assays
- Ag NP (metallothionein based)
- organic NP (antibody based)

ELISA
- specific antibodies raised
- sandwich ELISA developed for X-linked gelatine and β-lactoglobuline NP
WP3: Inorganic NP

**FFF-multidetector approach for Ag + SiO$_2$**

I) sample preparation
- extraction, digestion

II) asymmetric flow field-flow fractionation (AF$^4$)
- particle separation according to their size (1nm – few µm)

III) optical detection
- multi angle (MALS) and dynamic light scattering (DLS), UV-vis absorption
- particle detection (fractogram)
- size determination

III) inductively coupled plasma mass spectrometry (ICP-MS)
- elemental detection
- chemical identity quantification
- mass fraction

separation of particles from matrix material

S. Weigel, 5 Years Nanotechnology dialogue, Brussels 19 Oct. 2012
WP3: Inorganic NP

Single particle ICP-MS approach for Ag

- sp ICP-MS: element specific particle counting method
- addressing the EC recommendation for a definition in best possible way
- relatively easy to implement, use of existing instruments
- accepted as ISO work item (joint JWG2/3 project)
WP4: Organic NP

HPLC-MS

- Fullerenes ($C_{60}$, $C_{70}$) in food

HDC-UV-MALDI-ToF-MS

- size separation, characterization, identification of organic ENPs (fingerprinting)

DPPC: L-α-dipalmitoylphosphatidylcholine
DPPG: L-α-dipalmitoylphosphatidylglycerol
Part of the shell material of the Coatsome ENP

Coatsome

“Micelle A”

Cross-linked gelatine
Current status

- Most methods in validation phase/validated
- Standard Operation Procedures (SOPs) in preparation (will be available for download)
Current status

- **Interlab studies**
  - spICP-MS (full ILC)
    - Ag in food simulants: running
    - Ag in meat: scheduled April 2013
  - FFF (intra-project ILC)
    - SiO2, Ag: scheduled March 2013
- **Training workshops (hands-on) planned**
  - sp ICP-MS: March 2013
  - Multidetector FFF: April 2013
Conclusions and outlook I

- More information needed on actual applications of nanomaterials in the food sector
  - more targeted method development
- Suitable platform technologies for inorganic NP in food are EM, multidetector FFF, sp ICP-MS
- Organic NP are more difficult to measure due to their fragile nature and similarity of their building blocks with food components
Conclusions and outlook II

- Interaction of NP with food matrix still poorly understood
  - difficulties for matrix reference materials
- Sample preparation essential
- Sound method validation is crucial
  - certified reference materials
  - harmonised validation guidelines
- In the food area analytical methods not only needed for safety assessment, but also for product development and quality assurance
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