Analysis of silver nanoparticles in complex matrices

BfR Conference on Nanosilver, 8 Feb. 2012, Berlin

S. Weigel, R. Peters, H. Bouwmeester

RIKILT
WAGENINGEN UR

Current challenges

- Risk assessment based on
  - hazard characterisation (toxicology)
  - exposure assessment
  - Both requiring reliable analytical nanoparticle (NP) characterisation!
- The European Food Safety Authority (EFSA):
  "*The Scientific Committee makes a series of recommendations; in particular, **actions should be taken to develop methods to detect and measure ENMs [engineered nanomaterials] in food/feed** and biological tissues, to survey the use of ENMs in the food/feed area, to assess the exposure in consumers and livestock, and to generate information on the toxicity of different ENMs.*"

  (Scientific Opinion of the Scientific Committee on a request from the European Commission on the Potential Risks Arising from Nanoscience and Nanotechnologies on Food and Feed Safety. The EFSA Journal (2009) 958, 1-39)

Current challenges

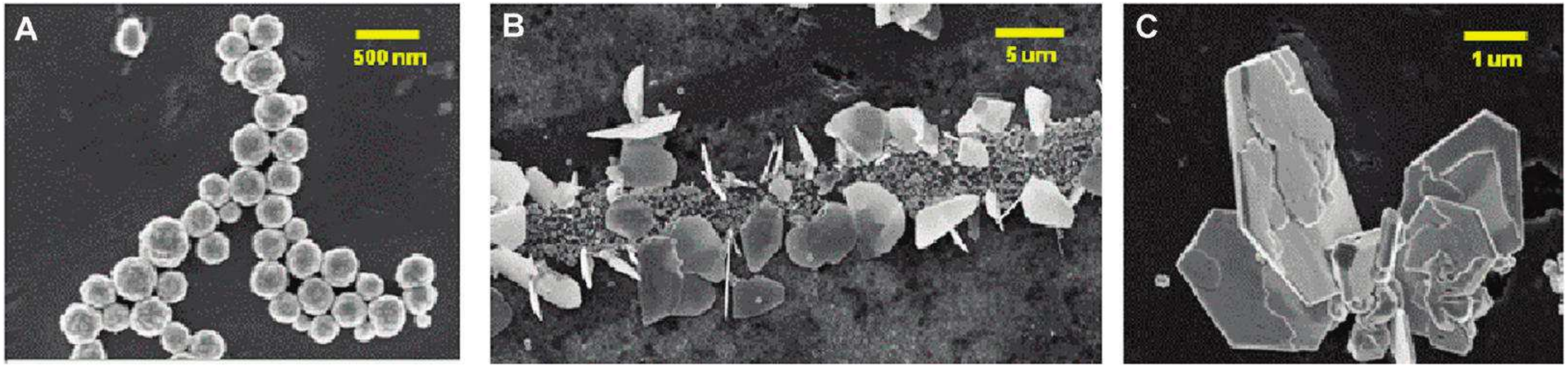
- Toxicological studies often without adequate analytical characterisation
    - Pure dispersion (non-specific methods – DLS, EM,..)
    - NP state in application medium (feed, cell culture medium etc.) not analysed
    - Characterisation in tissue, blood, media is non-specific, total Ag analysis (e.g. AAS)
    - no controls with ionic Ag
- Exposure assessment
    - Few size and composition specific methods for real matrices
    - Hardly any validated data on presence of NP in products/food

Current challenges

- Pure dispersions vs. real matrices
  - Various methods for pure particles/dispersions (EM, DLS, XRA, AFM, BET, Auger, ...)
  - In real matrices (biological tissues, food, consumer products) many methods not applicable or meaningless
    - agglomeration
    - binding of NP to matrix
    - presence of biogenic/geogenic NP

Current challenges

- Each particle type may behave differently
  - 20 nm ≠ 60 nm, spheres ≠ flakes, PVP ≠ citrate coated, 60 nm brand A ≠ 60 nm brand B,
  - different zeta potentials, production processes, aging



Liu et al., ACS Nano 4 (2010) 6903–6913

Current challenges

- EC definition of nanomaterial (2011/696/EU)
  - focus on number size distributions

> 2. 'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.

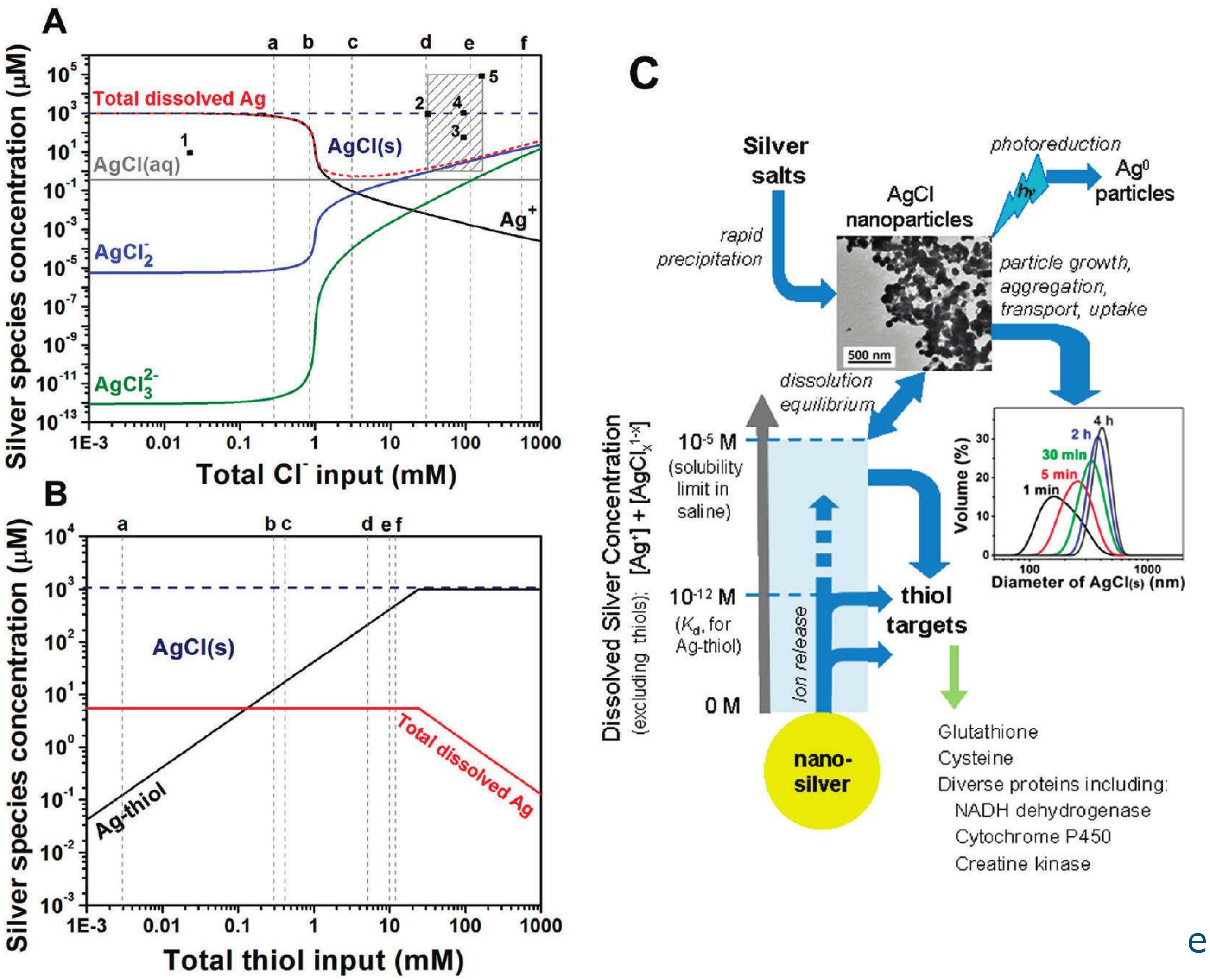
- Implications for analytical methods
  - particle counting methods
  - transforming mass into numbers is difficult

Current challenges

- Speciation
  - Particulate vs ionic Ag
  - Chemical state?
    - Ag^0
    - AgCl
    - Ag_2S
    - other NP (e.g. proteins) with attached Ag ions or clusters (Ag_n)
- Matrices typically containing salts (Cl^-), reducing sugars, acids, thiols (proteins) etc.

Current challenges

- Ag equilibria in complex systems



Liu et al., ACS Nano 4 (2010) 6903–6913

Current challenges

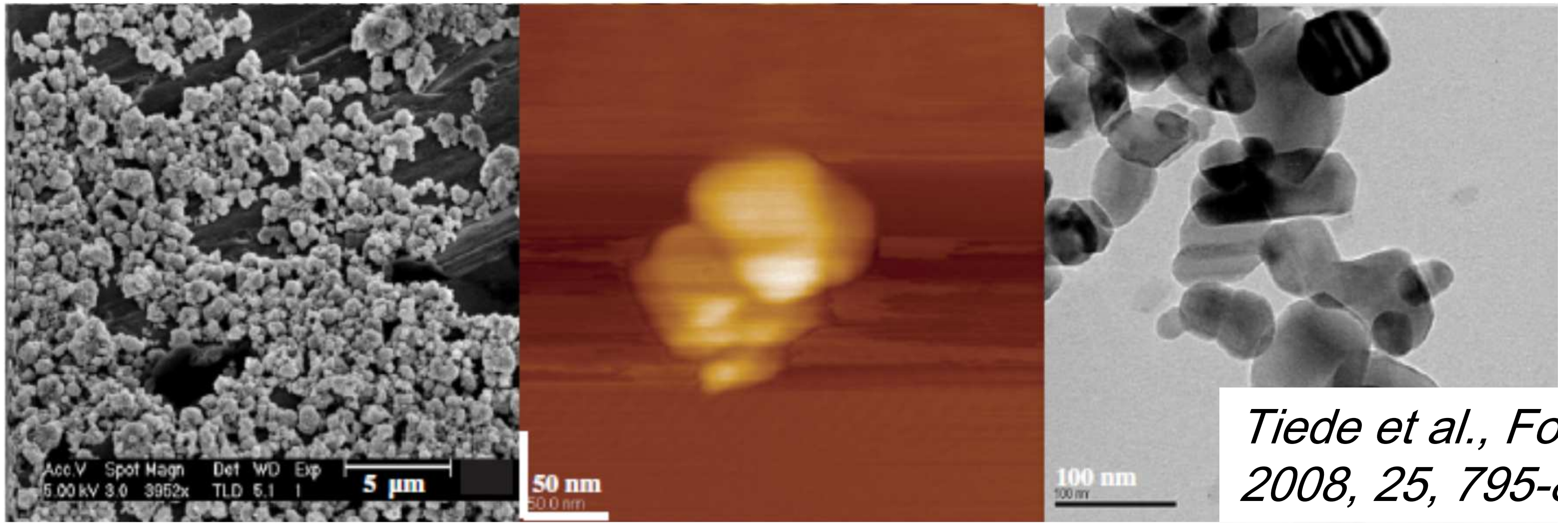
- More questions than answers!
    - objectively present the findings
    - name the limitations
    - don't draw premature conclusions
    - multiple lines of evidence

Approach

- Toxicology
  - size, shape, stability/agglomeration
  - chemical identity, speciation
  - in media, tissue
- Food & product monitoring
  - presence (yes/no)
  - identity: chemical composition, size (distribution)
  - concentration (mass, number)
  - ➔ Focus of EU project NanoLyse

Methods: Imaging

- Possibility to see your target analytes
- Determination of size and shape (+ ...)
- Various techniques
    - Scanning electron microscopy (SEM)
    - Transmission electron microscopy (TEM)
    - Atomic force microscopy (AFM)

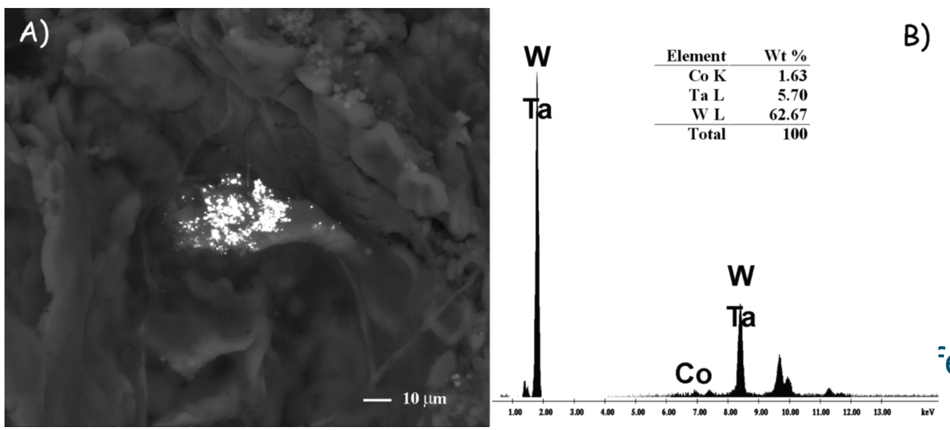


TiO_2 NP
a) SEM
b) AFM
c) TEM

Tiede et al., Food Add Contam 2008, 25, 795-821

Methods: Imaging

- Electron microscopy most frequently used for complex samples
- Various variants with specific advantages/ disadvantages available: WetSEM, ESEM, STEM, CryoTEM, ...
- Coupling to analytical tools, e.g. energy dispersive x-ray spectroscopy (EDX) -> elemental composition (semi-quantitative)



B)

ESEM-EDS

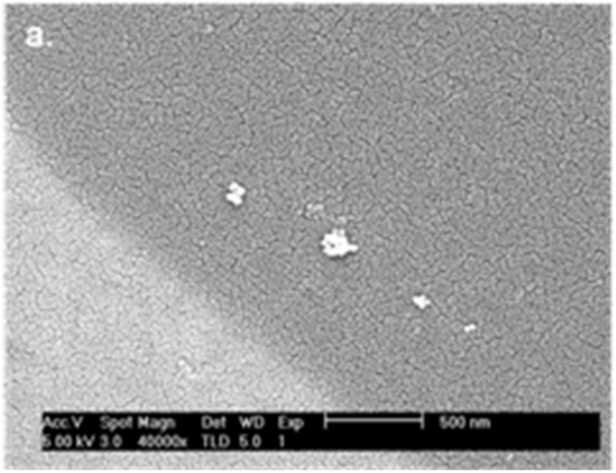
Bread sample from Sicily

Gatti et al., Crit Rev Food Sci Nutr 49, 275-282 (2009)

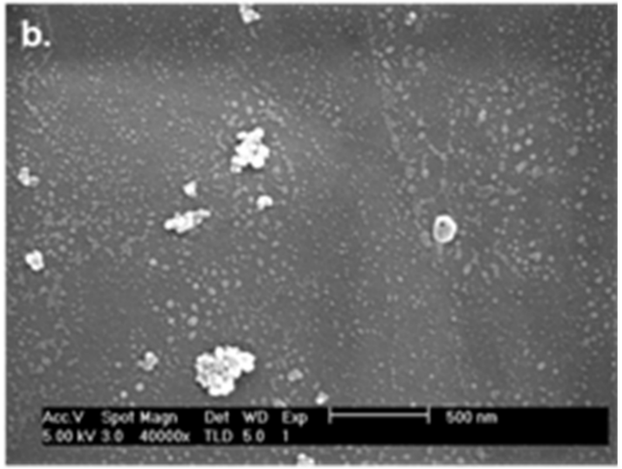
Methods: Imaging in food samples

- Formation of artefacts, matrix effects, ...
- Example: SEM of SiO_2

SiO2 in water



SiO2 in tomato soup

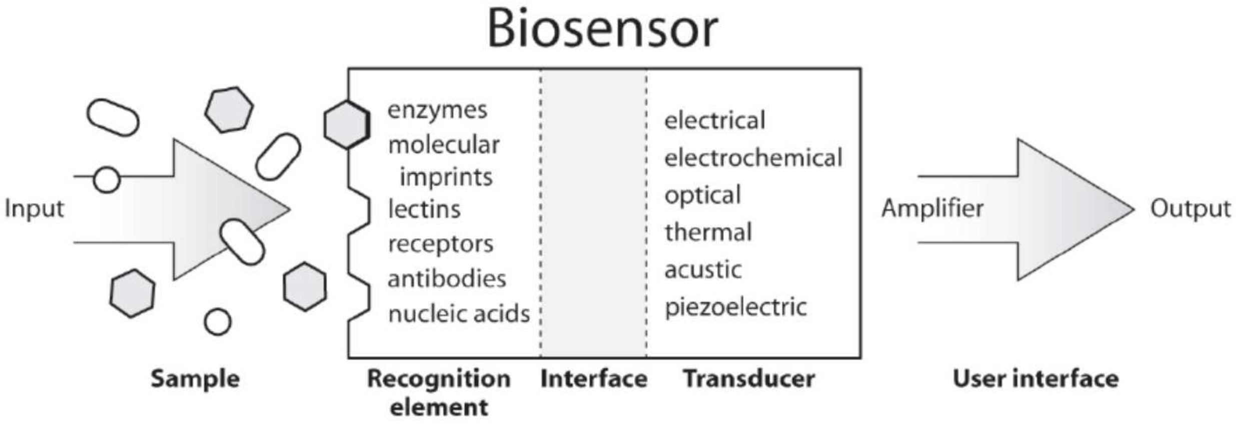


source: A. Dudkiewicz, FERA/NanoLyse

- Sample preparation critical!
  - Drying, ultracentrifugation, resin embedding, freezing, ...

Screening

- Rapid, cost-efficient detection of the presence of NP
- (Bio)Sensors

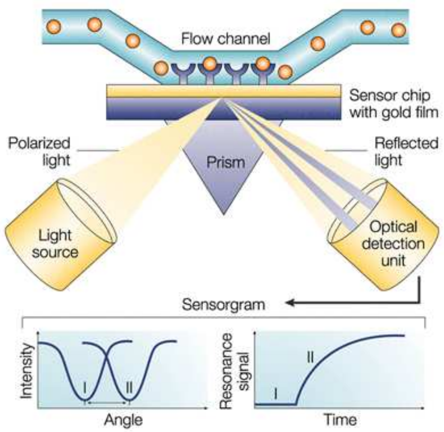


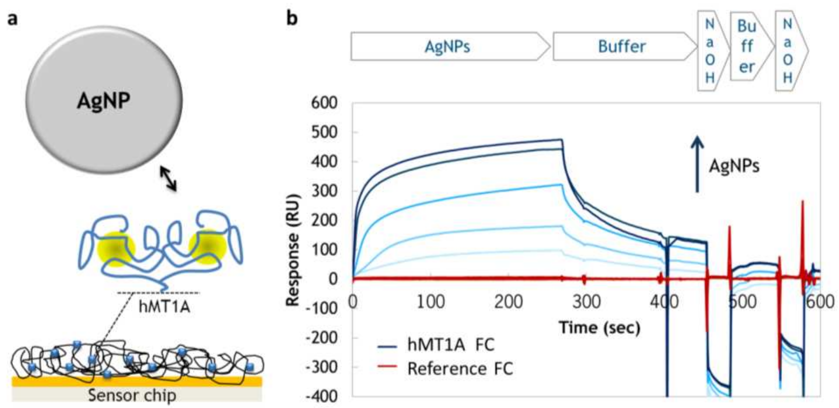
Chambers et al., Curr. Issues Mol. Biol. (2008) 10: 1-12

- Different platforms interesting for NP
  - Surface plasmon resonance (SPR)
  - Quartz crystal microbalance (QCM)

Screening

- SPR sensor for Ag NP
  - Recognition via metallothionin on chip
  - reversible binding – regeneration possible
  - sensitivity: µg/L range
  - applicable to food and environmental matrices





Rebe et al., Anal Bioanal Chem (2012) submitted

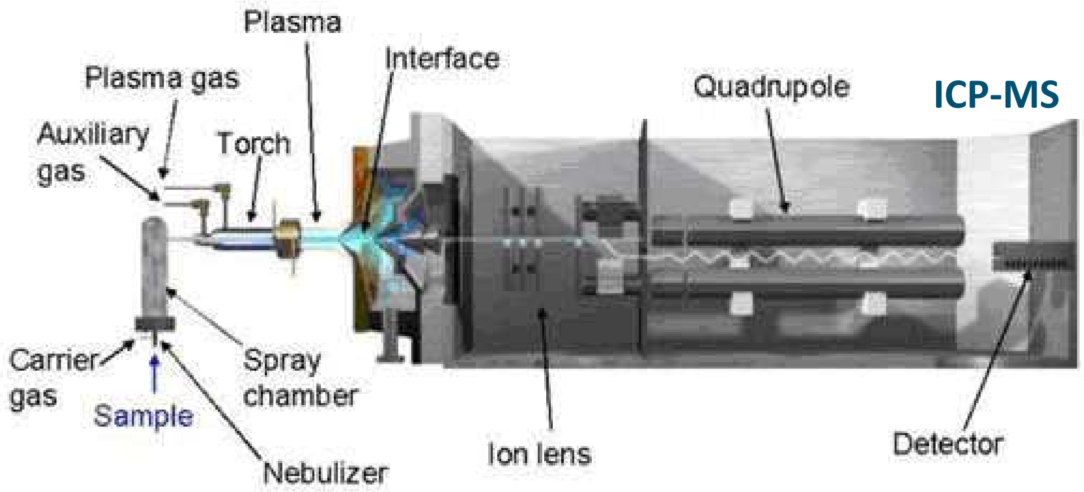
RIKILT WAGENINGEN UR

S. Weigel, BfR Conference Nanosilver, Berlin

NANOLYSE

Methods: Screening

- Total Ag determination
  - no Ag -> no Ag NP
    but: Ag present ≠ Ag NP present
  - Chemical total digestion of sample (e.g. microwave assisted aqua regia)
  - classical instrumental techniques: AAS, ICP-MS/OES, ...



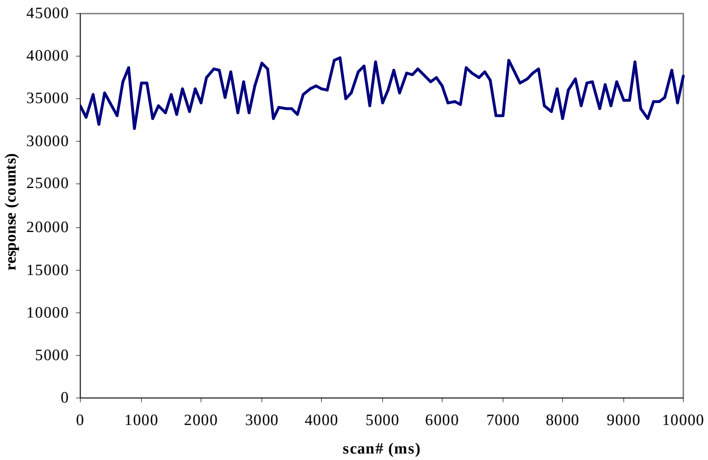
Methods: Light scattering (LS)

- Established technique, various variants
  - Static (SLS), multi angle laser (MALLS), dynamic (DLS)
- Widely used
- Severe limitations
  - Difficulties with polydisperse samples
  - Underlying algorithms not always clear/properly used
  - sensitive to matrix components
- Use as detector after size separation (e.g. FFF)

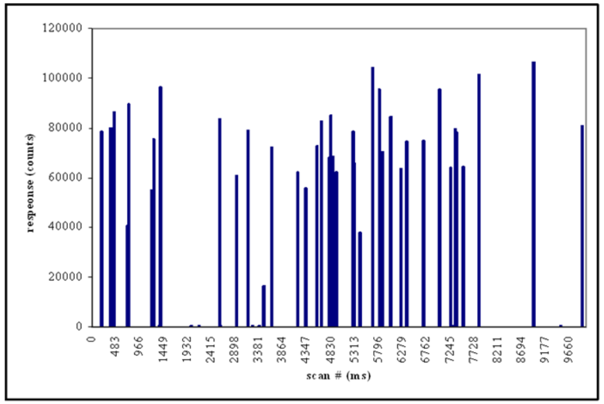
Methods: Single particle ICP-MS

- Principle: direct infusion of diluted samples into plasma

- conventional ICP-MS
  continuous signal
  integrated over time



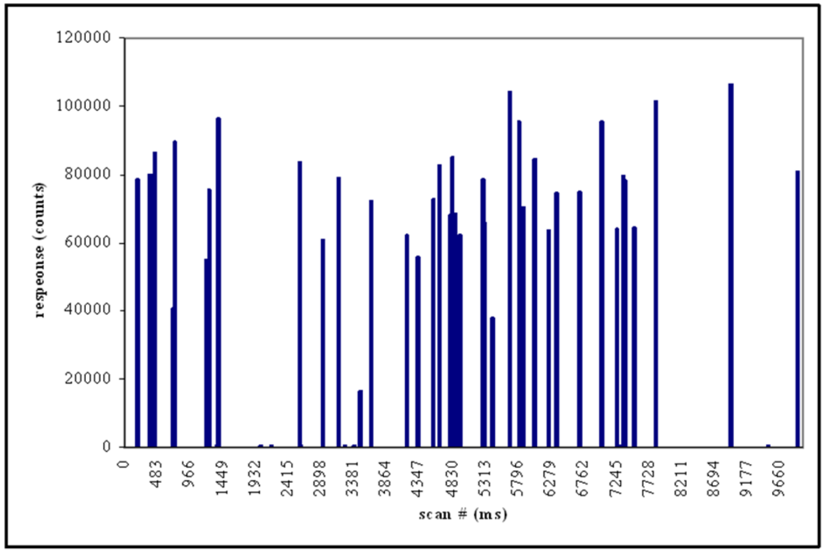
- sp-ICP-MS
  discontinuous signal
  heterogenous distribution of
  metal as NP, short time
  intervals



Methods: sp-ICP-MS

- The mass (~size) of the particles determines the intensity of the transient signals (peak height)

- The particle concentration determines the frequency of the transient signals (number of peaks min^{-1})



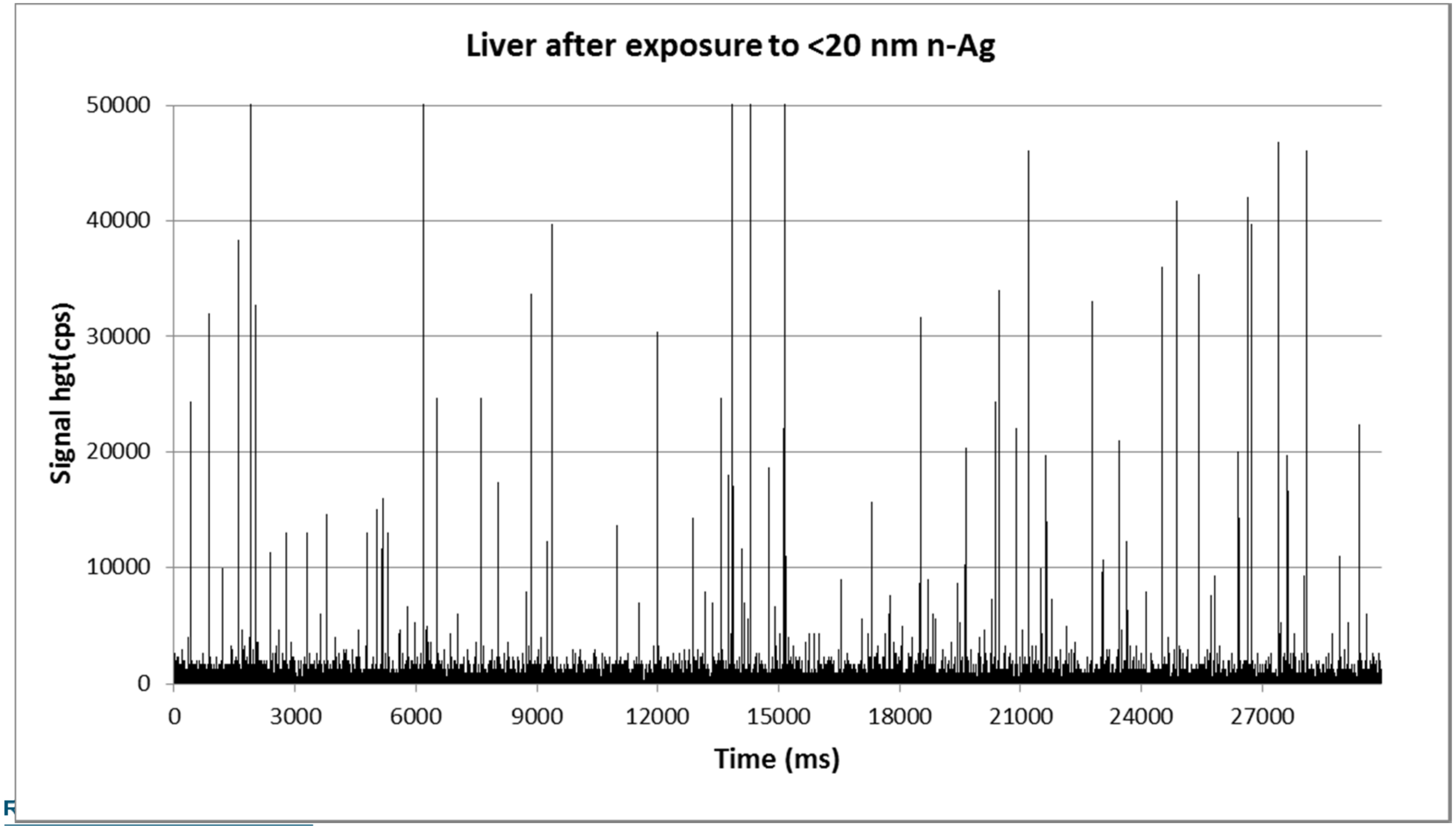
- minimal sample prep, often only dilution
- minimised NP interactions
- low detection limits (20 nm; 5 ng/L)
- counting particles

Exposure study using sp-ICPMS

- Pilot study with rats to examine the potential of AgNPs to cross the intestinal wall
  - Rats orally exposed to <20 nm, 50-60 nm AgNPs and $AgNO_3$ for 3 days.
  - Exposure dose 500 mg/kg bw via drinking water and custard
  - Blood and liver samples analysed using SP-ICPMS to determine bioavailability of AgNPs

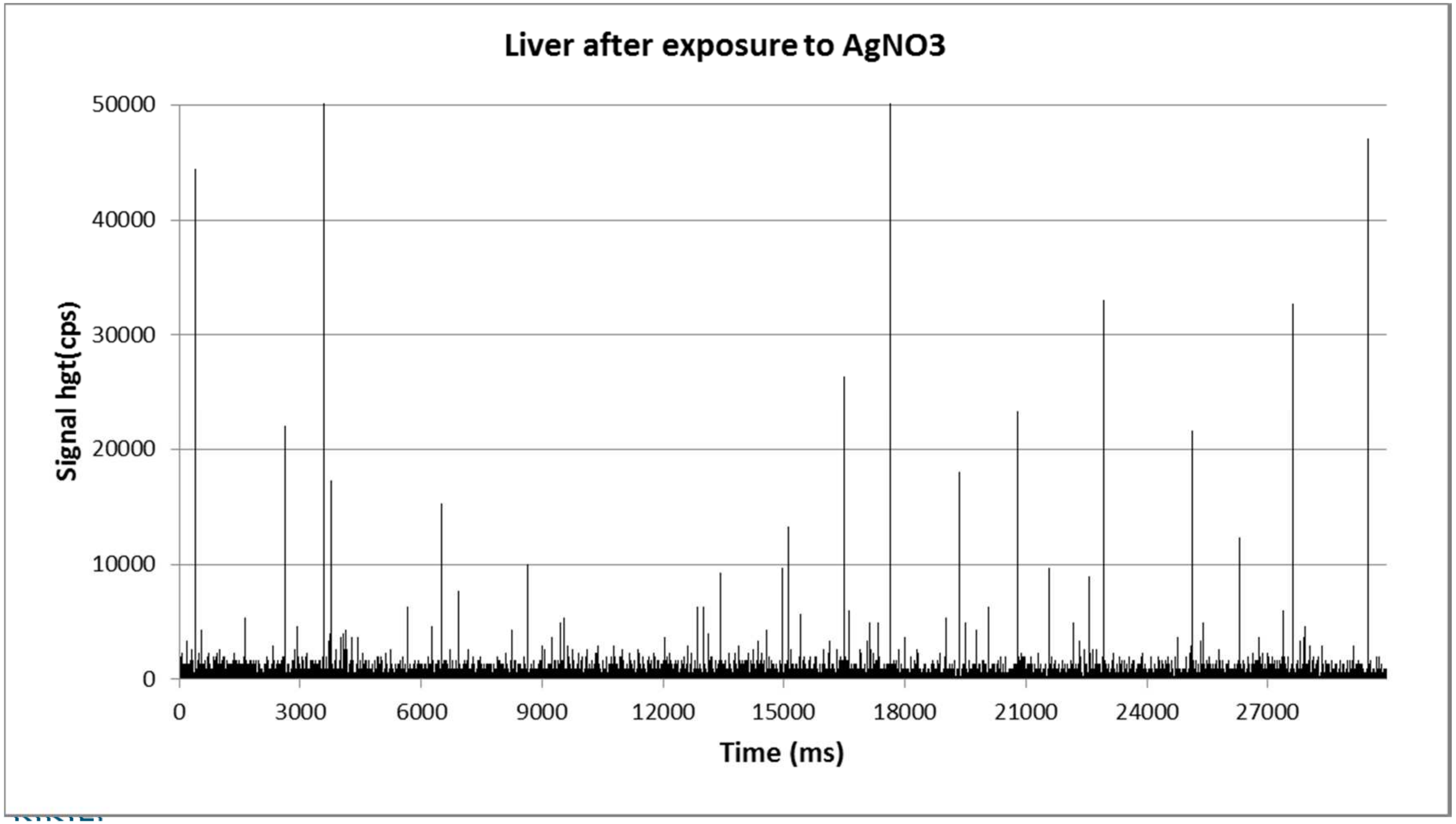
Example: Exposure study

Presence of AgNPs in liver after exposure to <20 nm n-Ag in food



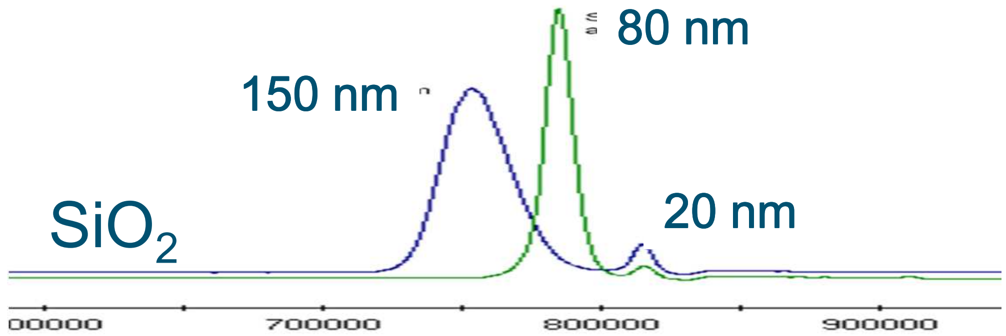
Example: Exposure study

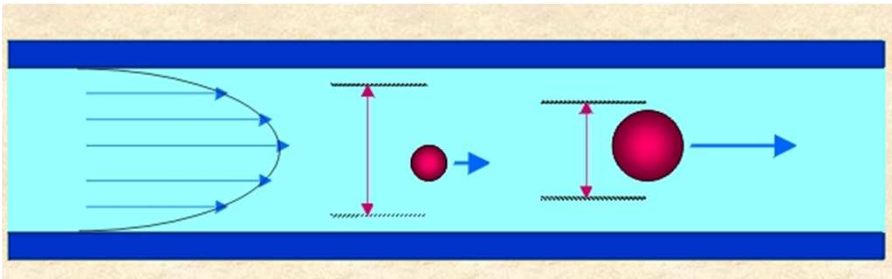
Presence of AgNPs in liver after exposure to AgNO3 in food



Methods:Hydrodynamic chromatography

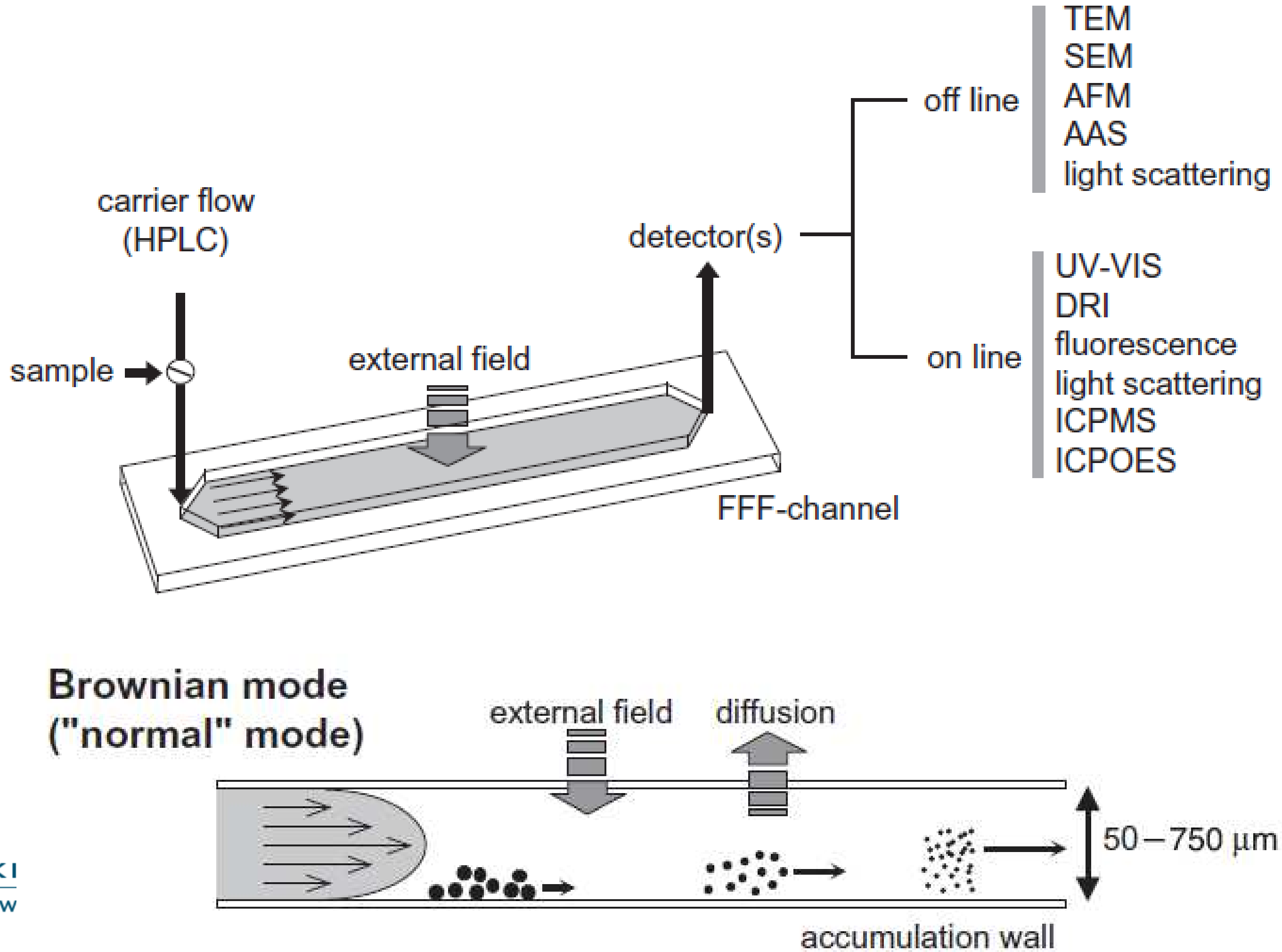
- Robust separation technique
- Compatible with standard HPLC equipment and detectors (UV/DAD, FLD, ICP-MS, ...)
- Wide dynamic range (<10 bis >1000 nm)
- Moderate resolution





Methods: Field flow fractionation (FFF)

- Most commonly used separation technique for NP

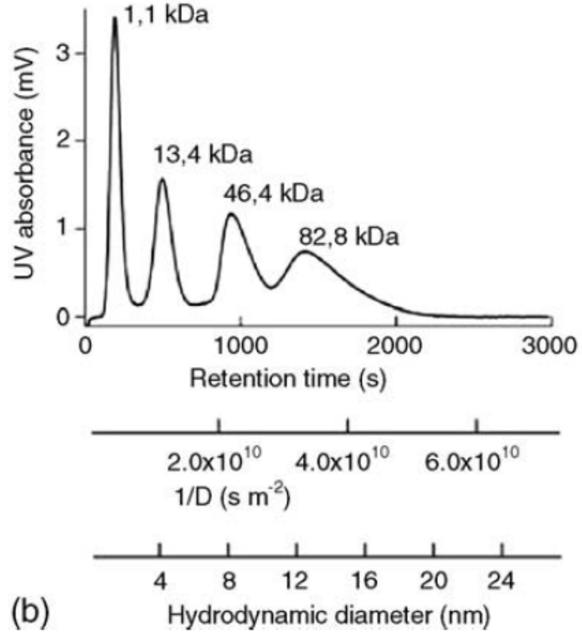


from: v.d. Kammer et al., TrAC (2011) 30:425-436

RIKI
W

Separation: Field-flow fractionation (FFF)

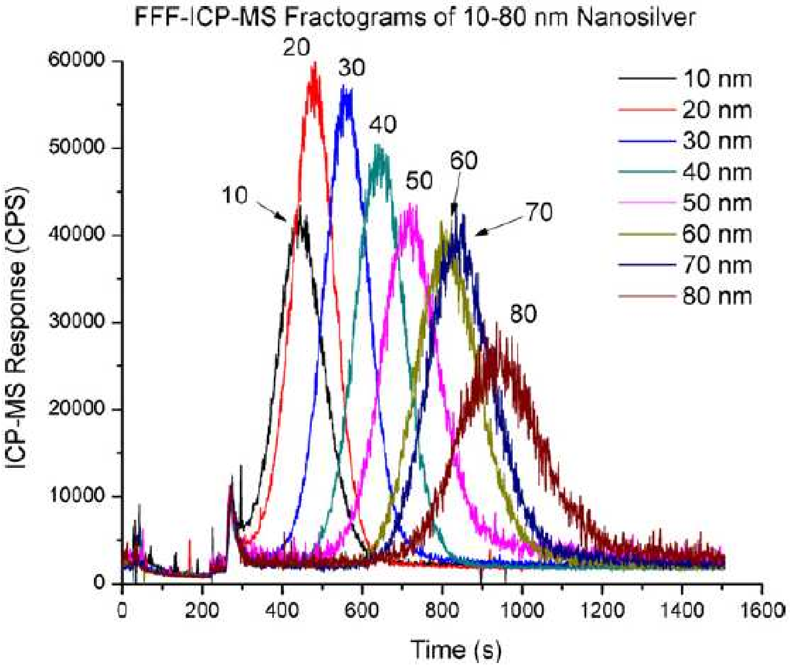
- Various options
  - Asymmetric flow (AF⁴)
  - Sedimentation (SedFFF)
  - Hollow fibre (HF⁵)
  - Electrical-, magnetic-, thermal-FFF
- Good resolution
- On-line coupling with MALLS provides size information
- Optimisation of several parameters crucial



Stolpe et al., Anal. Chem. Acta (2005) 535:109-121

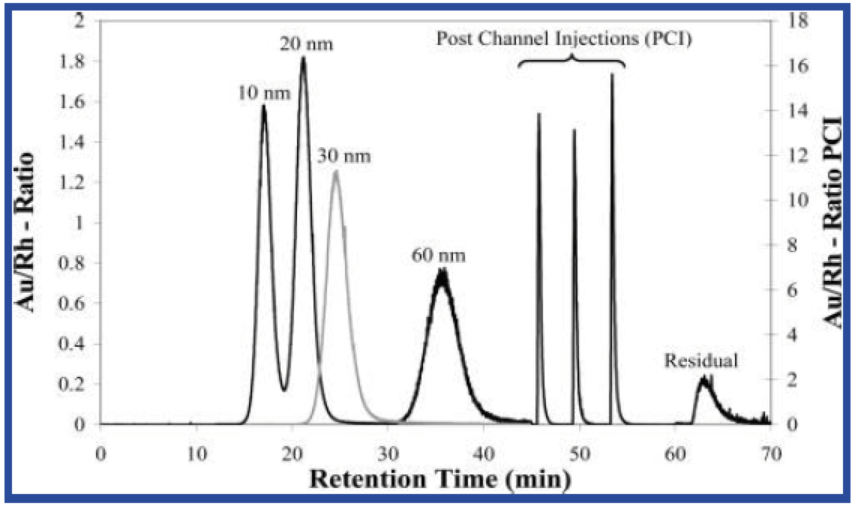
Methods: FFF-ICP-MS

- Coupling of FFF with ICP-MS provides element specificity



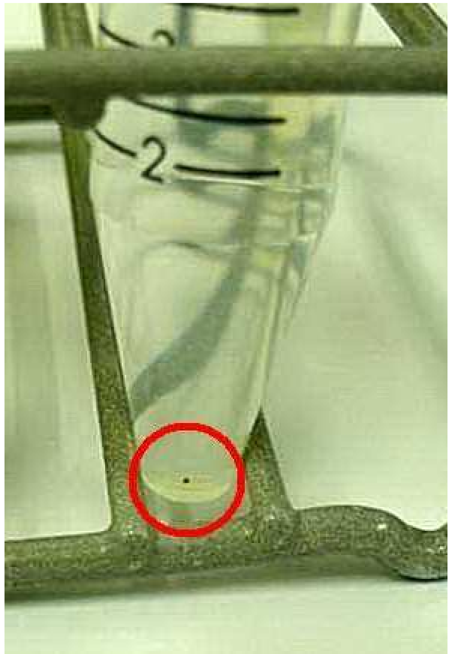
A.R. Poda et al. / J. Chromatogr. A 1218 (2011) 4219–4225

10 – 60 nm Au NP



Schmidt et al., Anal Chem 2011, 83, 2461-2468

Methods: Speciation



Ionic vs particulate Ag

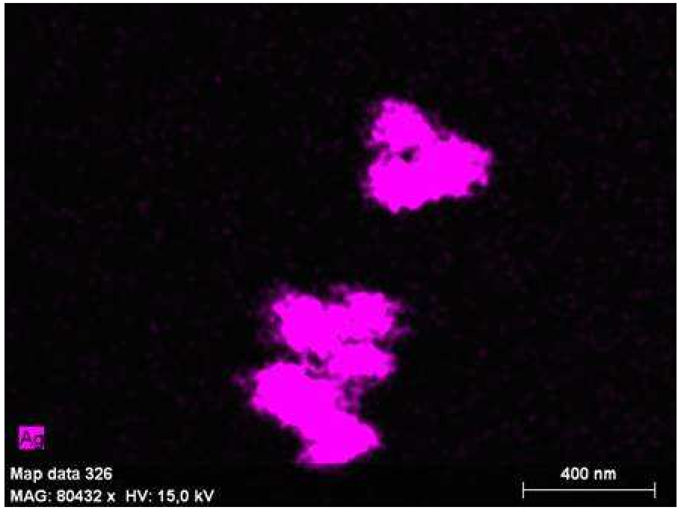
- No one fits alls solution
- Filtration, dialysis
- Centrifugation
- Size-exclusion chromatography
- total Ag – particulate Ag
- Electrochemical methods
- Difficulties: lower size range (<20 nm), continuous dissolution of certain Ag NP

Methods: Speciation

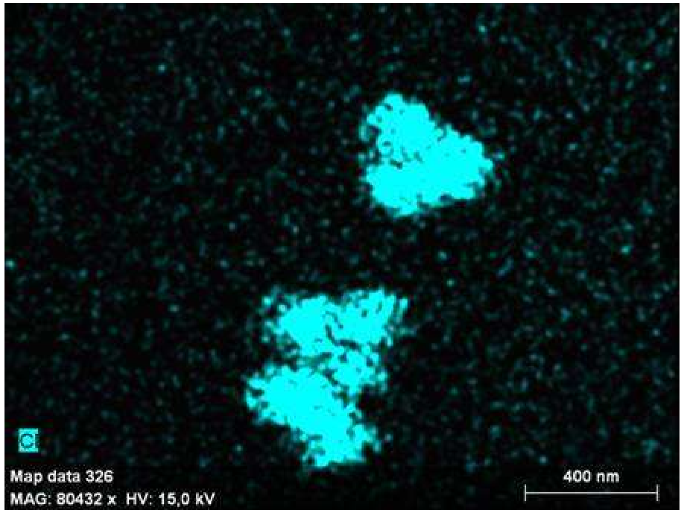
Elemental composition

- indirect approaches
- direct approach: EDX

SEM



Ag



Cl

RIKILT WAGENINGEN UR

Outlook

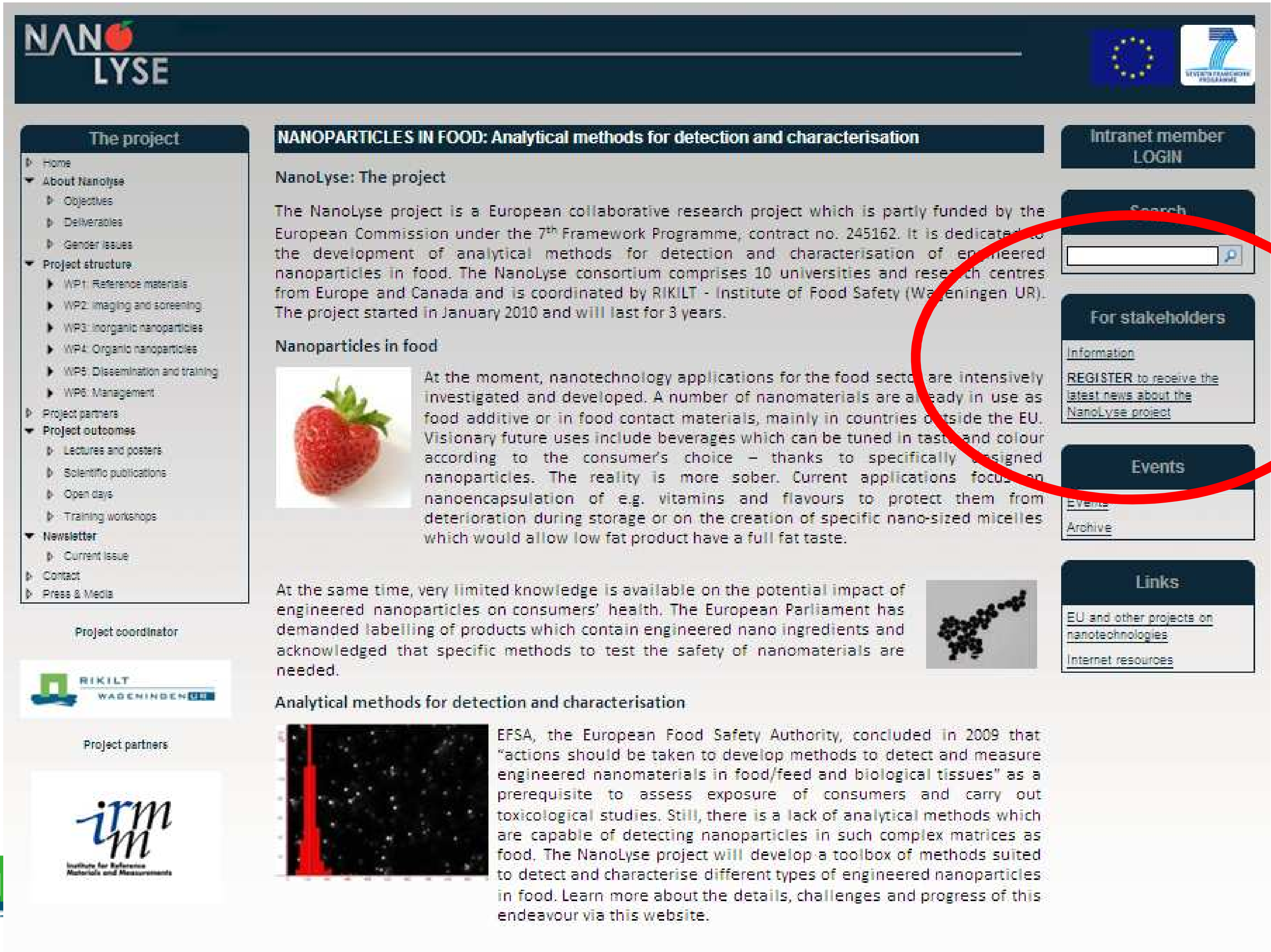
- Still urgent need for reliable analytical methods, especially for complex matrices
- Standardisation of methods?
  - Validation of methods
  - Standardisation of quality criteria
- Reference materials needed
- Real world: not academic monodisperse NP but industrial polydisperse impure NP

European collaborative research project

NANO LYSE

- Validated methods for ENP in food matrix
  - EM, sensors, sample prep, HDC, FFF, MS, LS
  - Inorganic NP: silver, silica, fullerenes ...
  - Organic NP: encapsulates, nano carrier systems
- Reference materials for ENP in food
- January 2010 – September 2013

www.nanolyse.eu



NANOLYSE

The project

- Home
- About Nanolyse
  - Objectives
  - Deliverables
  - Gender issues
- Project structure
  - WP1: Reference materials
  - WP2: Imaging and screening
  - WP3: Inorganic nanoparticles
  - WP4: Organic nanoparticles
  - WP5: Dissemination and training
  - WP6: Management
- Project partners
- Project outcomes
  - Lectures and posters
  - Scientific publications
  - Open days
  - Training workshops
- Newsletter
  - Current issue
- Contact
- Press & Media

Project coordinator

RIKILT WAGENINGEN UR

Project partners

irmm Institute for Reference Materials and Measurements

NANOPARTICLES IN FOOD: Analytical methods for detection and characterisation

NanoLyse: The project

The NanoLyse project is a European collaborative research project which is partly funded by the European Commission under the 7th Framework Programme, contract no. 245162. It is dedicated to the development of analytical methods for detection and characterisation of engineered nanoparticles in food. The NanoLyse consortium comprises 10 universities and research centres from Europe and Canada and is coordinated by RIKILT - Institute of Food Safety (Wageningen UR). The project started in January 2010 and will last for 3 years.

Nanoparticles in food

At the moment, nanotechnology applications for the food sector are intensively investigated and developed. A number of nanomaterials are already in use as food additive or in food contact materials, mainly in countries outside the EU. Visionary future uses include beverages which can be tuned in taste and colour according to the consumer's choice – thanks to specifically designed nanoparticles. The reality is more sober. Current applications focus on nanoencapsulation of e.g. vitamins and flavours to protect them from deterioration during storage or on the creation of specific nano-sized micelles which would allow low fat product have a full fat taste.

At the same time, very limited knowledge is available on the potential impact of engineered nanoparticles on consumers' health. The European Parliament has demanded labelling of products which contain engineered nano ingredients and acknowledged that specific methods to test the safety of nanomaterials are needed.

Analytical methods for detection and characterisation

EFSA, the European Food Safety Authority, concluded in 2009 that "actions should be taken to develop methods to detect and measure engineered nanomaterials in food/feed and biological tissues" as a prerequisite to assess exposure of consumers and carry out toxicological studies. Still, there is a lack of analytical methods which are capable of detecting nanoparticles in such complex matrices as food. The NanoLyse project will develop a toolbox of methods suited to detect and characterise different types of engineered nanoparticles in food. Learn more about the details, challenges and progress of this endeavour via this website.

Intranet member LOGIN

Search

For stakeholders

Information

REGISTER to receive the latest news about the NanoLyse project

Events

Events

Archive

Links

EU and other projects on nanotechnologies

Internet resources

Thanks for your attention

contact:

stefan.weigel@wur.nl

RIKILT
WAGENINGEN UR