



UNIVERSITÀ DEGLI STUDI DI PERUGIA Dipartimento di Medicina Veterinaria Corso di Laurea Magistrale in Scienze Biotecnologiche Mediche Veterinarie e Forensi

NANOTECHNOLOGY: A NEW FRONTIER IN FOOD

Giulia Canterini Maria Beatrice Gasparini Veronica Sperandio Monday December, 3rd, 2018 - 17:30

Aula 2 polo didattico di Medicina veterinaria



Dipartimento di Medicina Veterinaria Corso di Laurea Magistrale in Scienze Biotecnologiche Mediche Veterinarie e Forensi

NANOTECHNOLOGY: A NEW FRONTIER IN FOOD

Giulia Canterini Maria Beatrice Gasparini Veronica Sperandio

Outline

- 1. A current issue
- 2. We eat nanomaterials everyday..
- 3. Definition of nanotechnology and nanomaterials
- 4. Uses of nanotechnology in food industry
- 5. Most frequent nanomaterials in foods
- 6. The fate of food-related ENMs in the GIT
- 7. Toxicity and safety studies
- 8. Detection techniques to identify nanomaterials in food
- 9. Advantages/disadvantages
- 10. Future perspectives and conclusion

A current issue

Nanoparticelle negli alimenti e nel packaging: la tossicità è allo studio all'Istituto superiore di sanità

🌲 💿 9 maggio 2012 🛍 Sicurezza Alimentare 🔍 1 Commento





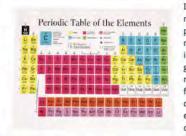
Le nanoparticelle sono sempre più diffuse nel settore alimentare: soprattutto nel packaging, ma anche nei cibi. Grazie a nuovissimi processi tecnologici, la materia viene strutturata in dimensione nanometrica (nm), ossia un miliardesimo di metro e utilizzato su substrati (per rendersi conto basta dire che 1 nanometro è 40mila a 80mila volte più sottile di un capello). Ridurre materiali come argento, ossido di titano e altri a queste nanodimensioni conferisce loro proprietà, fluidificanti, stabilizzanti, antibatteriche che non si trovano nel metallo o nel composto quando è utilizzato a dimensioni standard.

Additivi e ingredienti in formato nano servono a rendere le salse più fluide, il cioccolato più croccante e le preparazioni in polvere meno grumose oppure a prolungare la conservazione dei piatti pronti. Così, per esempio, la crosta del formaggio Brie diventa più candida con l'aggiunta di biossido di titanio (E171), un additivo usato fin dagli anni Sessanta, e che adesso è disponibile sotto forma di nanoparticelle; mentre il diossido di silicio (E551) nano rende fluido il ketchup.

In Europa triplica in numero di prodotti e di alimenti contenenti nanoparticelle

O 1 novembre 2010 Pianeta
 Commenti disabilitati
 Su In Europa triplica in numero di prodotti e di alimenti contenenti nanoparticelle



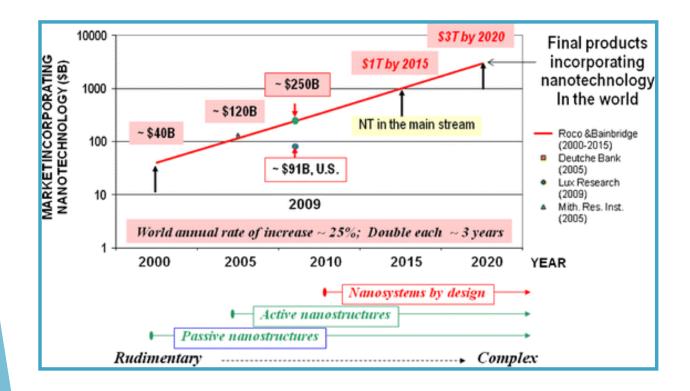


In Europa aumenta a dismisura il numero di beni di consumo e di prodotti che contengono nanoparticelle (particelle infinitamente piccole con un ordine di grandezza pari a un milionesimo di milimetro). Secondo una rilevazione firmata dal Beuc (Ufficio europeo delle organizzazioni dei consumatori) e dall'Anec l'anno scorso erano commercializzati nei punti vendita e in rete 151 beni di consumo. Un anno

dopo si è giunti a 475. Nella lista si trova di tutto, dai prodotti per bambini agli alimenti, dalle bibite ai cosmetici, dagli articoli per l'automobile alle apparecchiature elettriche. Ill Beuc si chiede qual è l'eventuale entità del rischio per gli utilizzatori. Stephen Russell, segretario generale dell' l'ANEC ritiene necessaria una legge specifica per rendere obbligatoria l'indicazione delle nanoparticelle sulle etichette dei prodotti. I consumatori chiedono anche la creazione di un registro europeo per le aziende che operano nel settore, con l'obbligo di indicare tutti i beni di consumo contenenti nanomateriali. Tra le richieste c'è anche l'ipotesi di rendere obbligatoria la tracciabilità delle nanoparticelle come ha proposto la presidenza dell'Unione europea.

- the aim to understand the possible toxicity
- arrive at worldwide applications about this field

Market timeline



 Refers to the projection for the worldwide market of finite products that incorporate nanotechnology

 doubling every 3 years as a result of successive introduction of new products containing nanomaterials

We eat nanomaterials everyday..



NIEHS

(National Institute of Environmental Health Sciences)





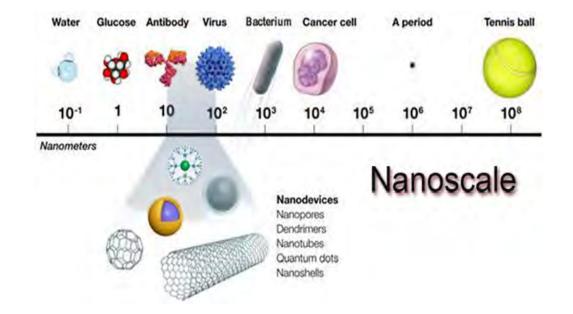
Control the power of engineered nanomaterials to improve public health Understand the potential risks associated with exposure to the materials

committed to supporting the **development** of nanotechnologies that can be used to improve products and solve global problems

Purpose of our work:

- 1. There is no single type of nanomaterial: Nanoscale materials can in theory be engineered from minerals and nearly any chemical substance.
- 2. The small size makes the material both promising and challenging: often seen as a "two-edged sword", properties that make nanomaterials potentially beneficial in product development and drug delivery, are the same properties that concern about the nature of their interaction with biological systems and potential effects in the environment.
- 3. Research focused on the potential health effects of manufactured nano-scale materials is being developed, but much is not known yet.

What is a "nanomaterial"?



"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". (2011/696/EU)

European Commission

What is nanotechnology?

promising technology

•

Nanotechnology involves

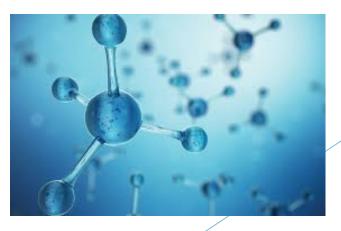




fabrication



dimension at least of 1-100 nm



SMALL

One nanometer is a **billionth of a meter!**

It's like comparing a millimeter with the distance between Milan and Taranto.

Ability to see and to control individual atoms and molecules.



Everything on Earth is made up of atoms

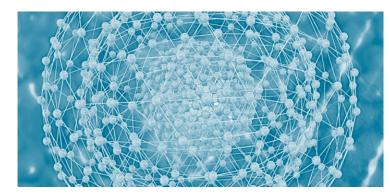




- higher strength
- lighter weight
- increased control of light spectrum
- greater chemical reactivity

scientists and engineers are finding a wide variety of ways to deliberately make materials at the nanoscale to take **advantage** of their enhanced properties

Sources



Engineered



Deliberately manufactured by humans to have certain required properties.

Incidental

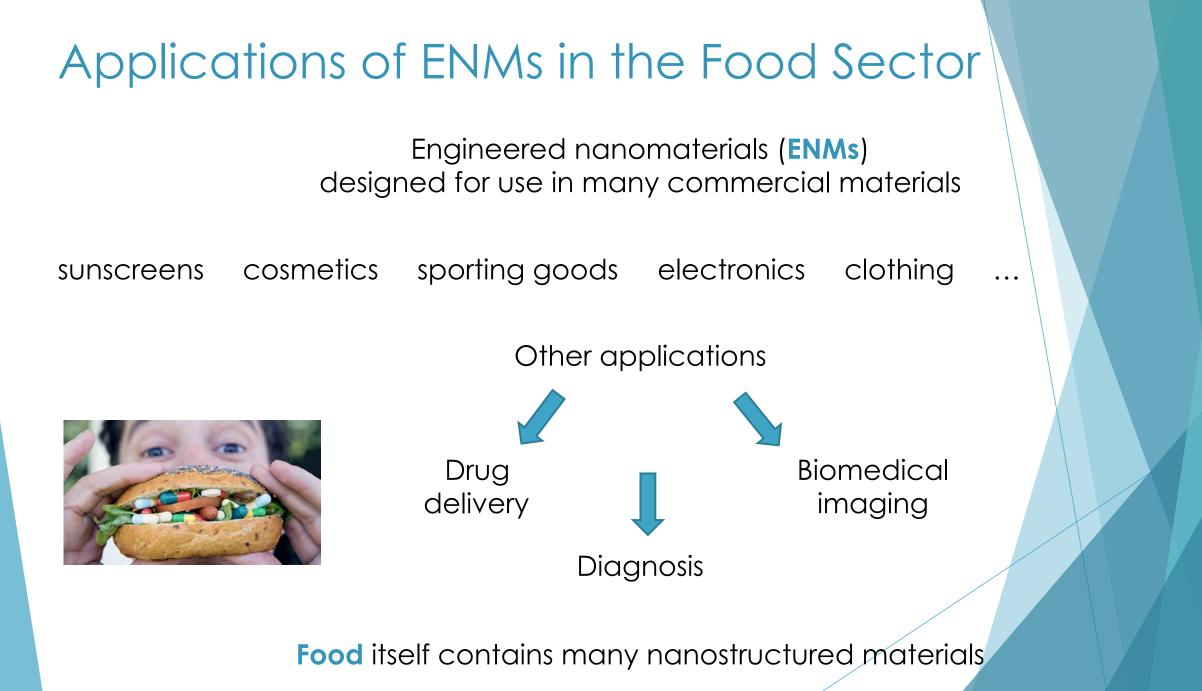


incidentally produced as a byproduct of mechanical or industrial processes.





occur through crystal growth in the diverse chemical conditions of the Earth's crust .



Nanotechnology in Food Processing

• Texture, Taste, and Appearance of Food

Nutritional Value (Encapsulation)



 Preservation or Shelf-Life (slowing down degradation processes)

Food Packaging

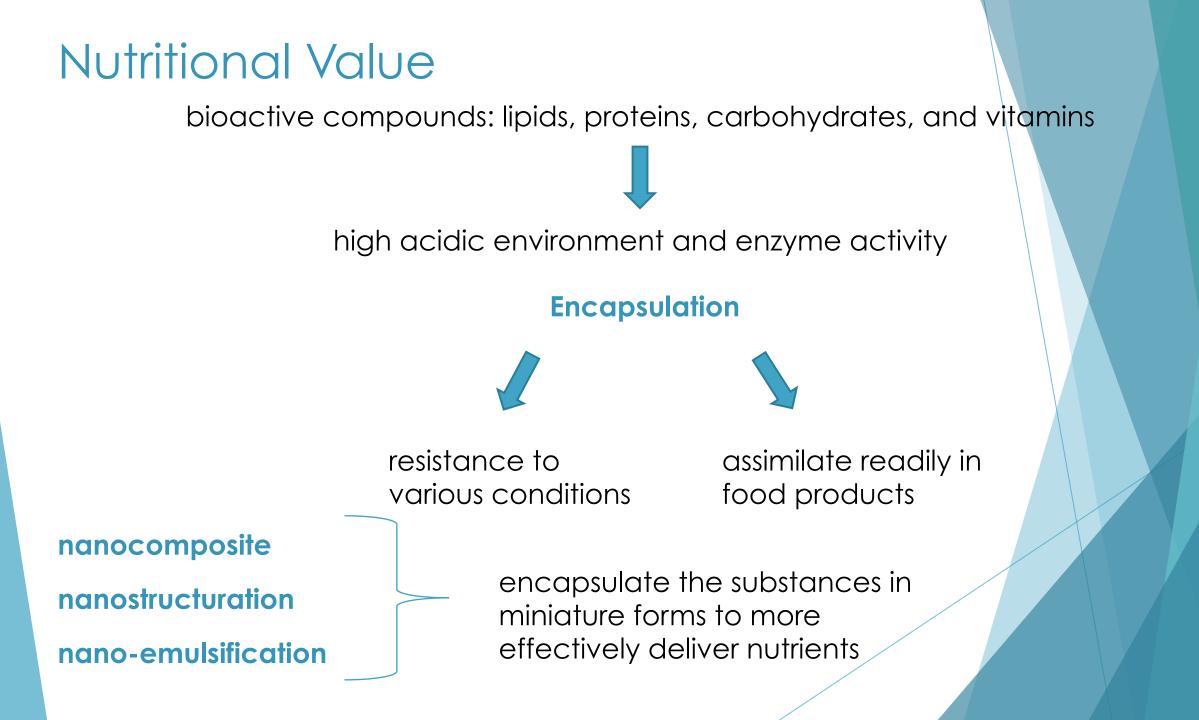
Texture, Taste, and Appearance of Food

improve the food quality and also helps in enhancing food taste.

Nanoencapsulation techniques

improve the flavor release and retention and to deliver culinary balance.

Nanoparticles provide promising means of improving the bioavailability of nutraceutical compounds due to their subcellular size leading to a higher drug bioavailability.



Preservation or Shelf-Life

Nanoencapsulation



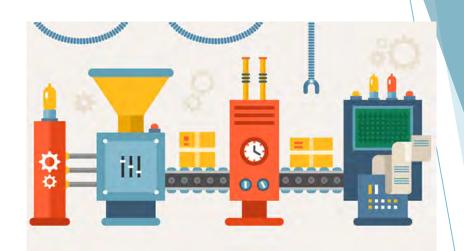
extends the shelf-life of food because slowing down the degradation processes or prevents degradation until the product is delivered

Edible nano-coatings

barrier to moisture and gas exchange and give improvements that could also increase the shelf-life of manufactured foods

Food Packaging

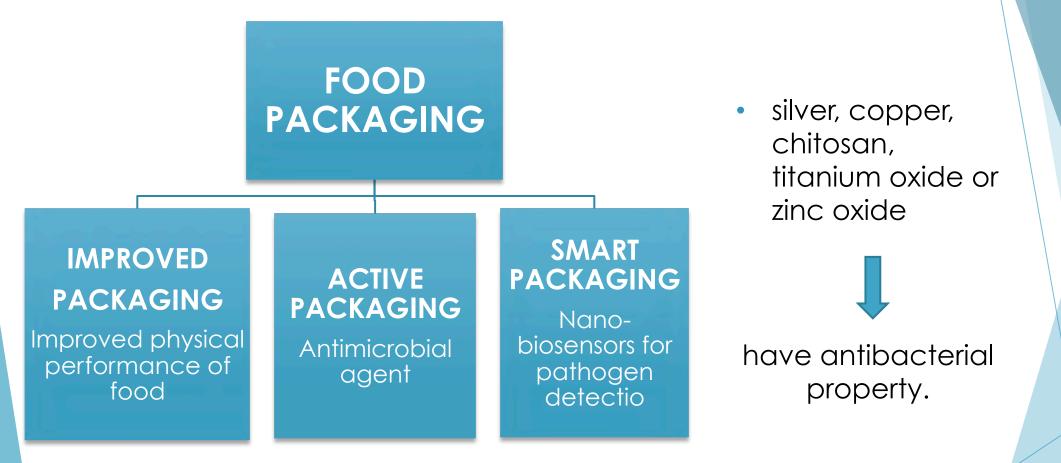
A desirable packaging



gas and moisture permeability combined with strength and biodegradability

Application of nanocomposites as an active material for packaging and material coating can also be used to improve food packaging

Food Packaging



- Not limited to antimicrobial food packaging
- Barrier from extreme thermal and mechanical shock.

Applications of ENMs in the Food Sector

Area of application	Application	Reference
Agriculture (Nano-modification of seed and fertilisers/pesticides)	Pesticides	[27]
	Targeted genetic engineering	[29]
	Preservation	[29,30]
	Agrichemical delivery	[29]
	Sensors to monitor soil conditions	[31]
Processing (Interactive smart food)	Nanoencapsulation of flavors/aromas	[32-37]
	Nanoemulsions	[38]
	Anti-caking agents	[39]
Nutrition (Food fortification and modification)	Nutraceuticals	[40-42]
	Nutrient delivery	[32,41]
	Mineral and vitamin fortification	[43.44]
	Drinking water purification	[45]
	Sensory characteristics of supplements	[46-51]
Products (Smart packaging and food tracking)	UV protection	[52-54]
	Antimicrobials	[55-63]
	Condition and abuse monitors	[46.47.64]
	High barrier plastics	[65-68]
	Security	[45.69,70]
	Contaminant sensors	[51.71-75

Table 1. (Potential) applications of nanotechnology in food science.

four key focus areas

Applications of ENMs in the Food Sector

- The potential for food nanotechnology applications
 seems unlimited.
 - Result in promising applications for improved food production, processing, packaging and storage
 - major focus

processing and formulation of food ingredients to form nanostructures that are claimed to offer improved taste, texture and consistency

Most frequent nanomaterials present in food

Nanomateriali	·	Matrici
Inorganici		Mangimi
Biossido di titanio, TiO ₂	E171	Integratori (consumo animale)
Ossido ferrico/ferroso, Fe ₂ O ₃ /Fe ₃ O ₄	E172	Alimenti (consumo umano)
Argento metallico, Ag	E174	Integratori (consumo umano)
Oro metallico, Au	E175	Simulanti alimentari
Silice amorfa, SiO ₂	E551	(migrazioni da materiali a contatto con gli alimenti)
Ossido di alluminio (allumina), Al ₂ O ₃		A Etanolo 10%
Ossido di zinco, ZnO	· · _ · · · ·	B Acido acetico 3%
Ossido di cerio, CeO		C Etanolo 20%
Nanoargille		D1 Etanolo 50%
Nanotubi e fullereni		D2 Olio vegetale
Organici		Sostitutivi D2 Etanolo 95%, isoottano
Nisina	E234	E poli(ossido di 2,6-difenil-p-fenilene)
Chitosano		
Nano-incapsulati (micelle, liposomi e dendrimeri)		
Compositi		
Nanoargille funzionalizzate		
Nanoparticelle metalliche funzionalizzate		
Nanocompositi a matrice polimerica	1	

Nanotitanium (E171)



Consumed annually at about **4 million tons** worldwide.

Used in **paints**, **coatings**, cosmetics , nutritional supplements and food products, e.g., **colorants**.

Reduces E. coli contamination of food surfaces.

FDA approved TiO2 as a food color additive with the stipulation that the additive should not to exceed 1% w/w. TiO2 was also approved by the US FDA in food packaging.



- Potential to penetrate the blood-brain barrier, BUT evidence is lacking that could link systemic responses to translocation of particles to target sites.
- Study conducted by Gerhard Rogler TiO2 particles are able to penetrate the intestinal epithelial cells leading to inflammatory processes
- In Italy Study of 4 years ago demonstrated that the administration in low doses of titanium bioxide in rats is able to induce alteration of ovary and of testosterone metabolism.

Ferrous-ferric oxide (E172)

Iron oxide and hydroxide are colorants of variable color of mineral origin. They can be contained in chewing-gum, confetti and sweets.



- ► These NPs **bioaccumulate** in the liver.
- In vivo studies have shown that after entering the cells, iron oxide NPs remain in endosomes/lysosomes, release into cytoplasm after decomposing, and contribut to cellular iron pool.

These NPs exert their toxic effect in the form of **cell lysis**, **inflammation**, **and disturbing blood coagulation system**.

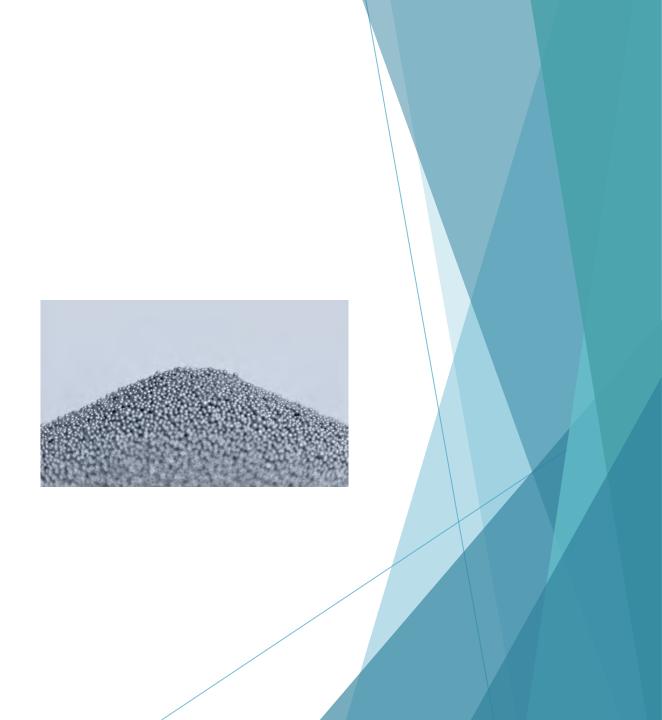


Metallic silver (E174)

390 products

textiles, cosmetics/hygiene products, cleaning agents (detergents, soaps), kitchen supplies, toys and building materials (glues).

Highly toxic to mammalian cells and human health: damage brain, induce oxidative stress. At high doses, it has been shown to cause neurotoxicity in rats and mice.



Aluminium oxide

• Contained above all: in **chemical yeast**, in **melted cheeses**.

• One of the most used materials. It is characterized by an excellent **thermal conduction**. But, **not for storage**!!!

• Cereals and derivatives, vegetables (spinach, horseradish and lettuce), mushrooms, drinks.



Aluminium oxide

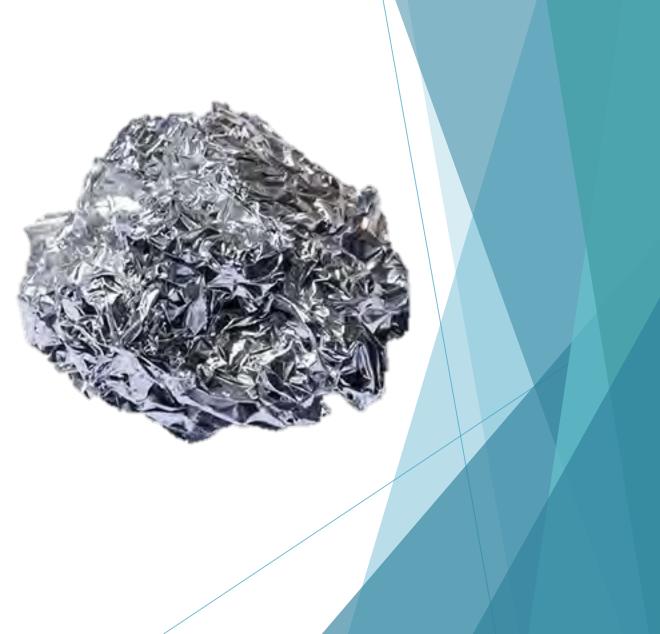
•EFSA has restricted the dietary intake of aluminum to 1mg / kg of body weight per week.



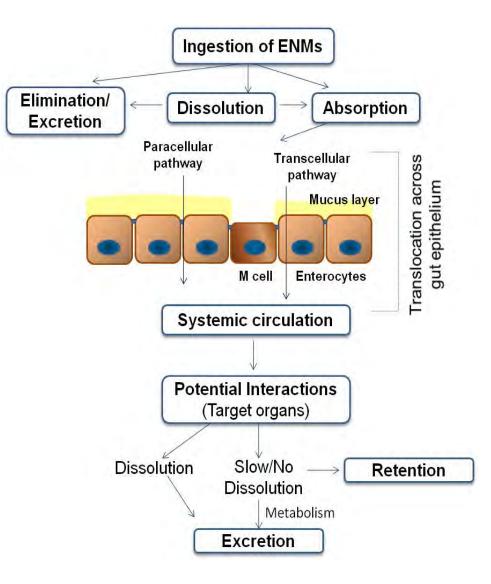
muscle weakness, bone pain,

osteoporosis, growth retardation in children. Worsening of mental abilities, **dementia** and

occur individuals convulsions mainly in with



The fate of food-related ENMs in the GIT



Factors that influence the biological availability of an administered NP:

- Particle size
- Surface area
- Particle number
- Aggregation/agglomeration state
- Surface charge
- Shape
- Aspect ratio

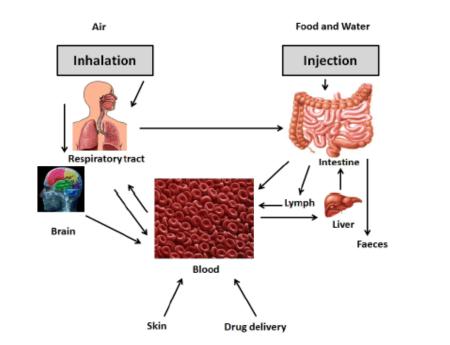
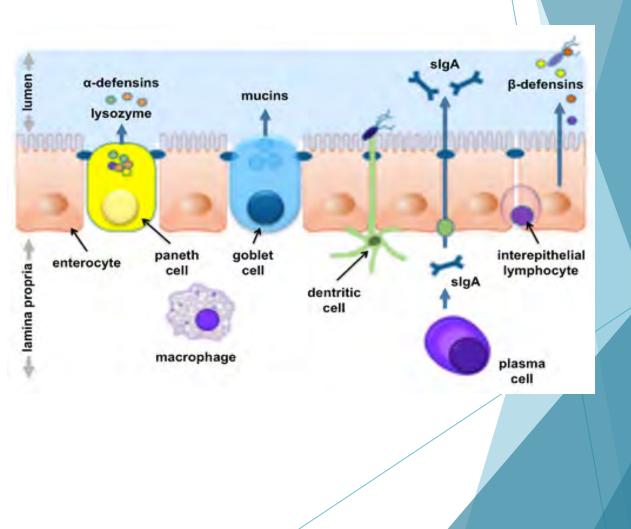


Figure 3: Illustration of possible routes of nanoparticles uptake, allocation and excretion in humans.

• Intestinal barrier

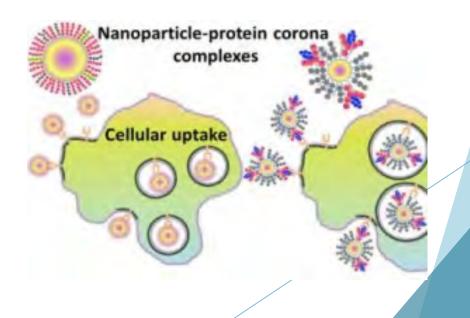
- Multistep process
- diffusion through the mucus layer
- contact with enterocytes and/or M-cells
- uptake. The most common mechanism for uptake of NP into intestinal epithelial cells appeared to be endocytosis.



Net neutral or positive
 surface charge

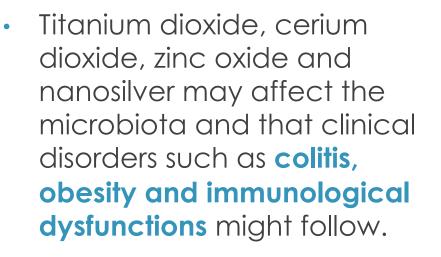
prevent mucoadhesion, favoring penetration

 Negatively charged hydrophilic and lipophilic compounds hindered. NP will develop a corona of adsorbed proteins, small molecules, and ions. The effects of the protein corona are variable. In some cases, cytotoxicity is reduced.



The interaction with the Gut Microbiota...







• **Iron** nanoparticles may show advantages over traditional iron-based supplemental treatment because they do not interfere with the microbiota, and some NMbased therapeutic interventions might be employed for treating intestinal infections.

Toxicity and safety studies



SolNanoTox project

two different classes of MNMs will be investigated:

- 1. titanium dioxide as
 - an example for insoluble species due to its stability in water

2. aluminium

representing the soluble category.

Aluminum nanoparticles form aluminum ions, whilst titanium dioxide nanoparticles may cross the intestine as intact nanoparticles.

Different target organs and toxicity for the two NMs.



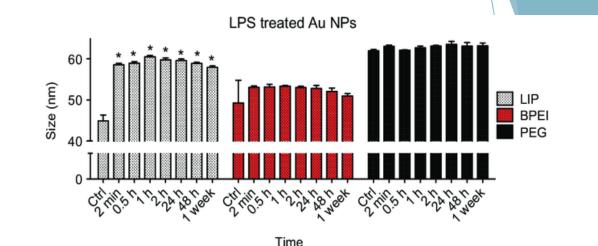
Cellular toxicity:

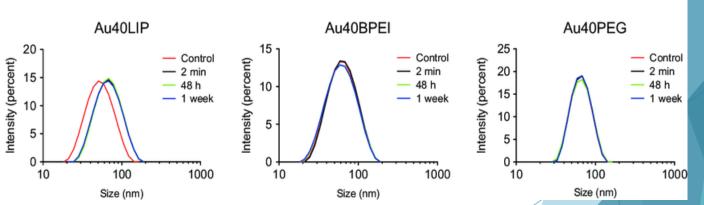
- The cell uptake, ROS level, cell cycle and aopoptosis were determined for toxicity studies in human gastric epithelial cell GES-1 and colorectal adenocarcinoma cell Caco-2.
- 24 hours exposure time and 100 micrograms of concentration is safe for both cell lines as silica NPs do not pass through Caco-2 cell monolayer after 4 hours. These NPs have low capability to cross the gastrointestinal tract in vivo while long term effect should be considered.



- SiO₂ nanoparticles stimulate the proliferation of human colon carcinoma cells (HT29) causing interference with MAPK/ERK1/2 signaling pathway.
- TiO2, ZnO, Au and SiO2, upon their absorption and passage across the GIT adsorb calcium ions and LPS and the resulting NPs-calcium-LPS conjugates

activate both peripheral blood mononuclear cells and intestinal phagocytes.



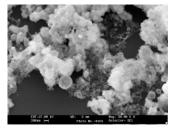


Gastrointestinal disorders:

- Crohn's disease
- Obesity
- IBD
- Ulcerative colitis
- Gastric and colorectal cancer

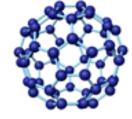


Nanoparticles: what makes them different



- Large specific **surface**
- Chemical reactivity very different compared to bulk material
- Quantum effects lead to special properties (electronic, mechanical, optical ...)
- Matrix dependent properties
- Many forms: fullerenes, nanotubes, nanocarriers, nanoemulsions, -encapsulates





Nanomaterials characterization issues

For what

- Optimisation for production and quality assurance
- Characteristics for specific applications
- Interaction with biological systems
- Behaviour in the environment

Driving force: safety assessment

- Possible health effects
- Environmental effects (transport, fate, interaction with living organisms)
- Knowledge gaps concerning NM characteristics (SCENIHR...)
- Regulation of nanomaterials (REACH, food, cosmetics, ...)

Interaction of NM with biological matrices

Consequences of phys.-chem. Properties

- Thermodynamically unstable or metastable
- Aggregation or agglomeration
- Interaction with surrounding matrix
- Ageing
- Adsorption of ions surface charge
- Nuclei for heterogeneous crystallisation
- Catalytic effects



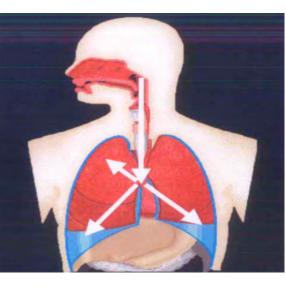
- Interaction with functional groups of biopolymers
- Formation of reactive oxygen species
- Nuclei for induced crystallisation

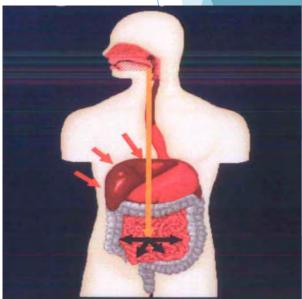
Effects

- Translocation from portal of entry to target organs
- Protein binding
- Cellular uptake
- Accumulation and retention
 - Cell/tissue response







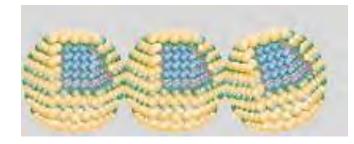


Detection techniques for identification of nanomaterials

• A number of tools – no best techniques

Issues

- State of the material
- Sample preparation
- Measurement protocols
- Particle environment
- Laboratory vs routine measurements





Detection techniques for identification of NMs

Nanoparticle Tracking

Ensemble analytical techniques

- Dynamic Light Scattering (DLS) (= Photon Correlation Spectroscopy, PCS)
- Laser Diffraction/Static Light Scattering
- Low Pressure Impactor (LPI) Electrical Low Pressure Impactor (ELPI)
- Scanning Mobility Particle Sizer
- Differential Mobility Analyser (DMA)
- Field Flow Fractionation (FFF)
- Centrifugal sedimentation
- Specific Surface Area (BET)
- Time of Flight Mass Spectroscopy (ToF MS)

Single-particle techniques

- Scanning Electron Microscopy (SEM)
- Transmission Electron Microscopy (TEM)
- Field emission gun scanning/transmission Electron Microscopy (FE STEM)
 - Energy Dispersive Spectrometry (EDS)
 - Electron Energy Loss Spectorscopy (EELS)
- Atomic Force Microscopy (AFM)

Microscopy and microscopy-related techniques

TEM

Optical

CRT

SEM

The typical dimensions of nanoparticles are below the diffraction limit of visible light, so that they are outside of the range for optical microscopy.

The most popular tools:

- atomic force microscopy (AFM)
- scanning electron (SEM)
- transmission electron microscopy (TEM)

Nanoparticles can not only be visualized, but also properties such as the state of **aggregation**, **dispersion**, **sorption**, **size**, **structure and shape** can be observed.

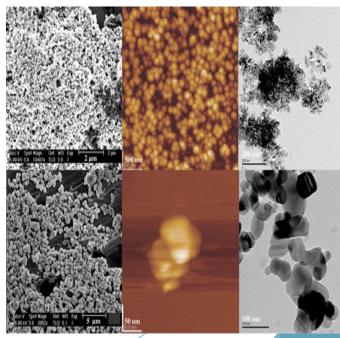
Transmission Electron Microscopy (TEM)

Advantages

- Accessible size < 1 nm, very high resolution
- Direct Method
- No calibration necessary
- Can be directly combined with analytical methods (e.g. EELS)
- Any particle shape is accessible

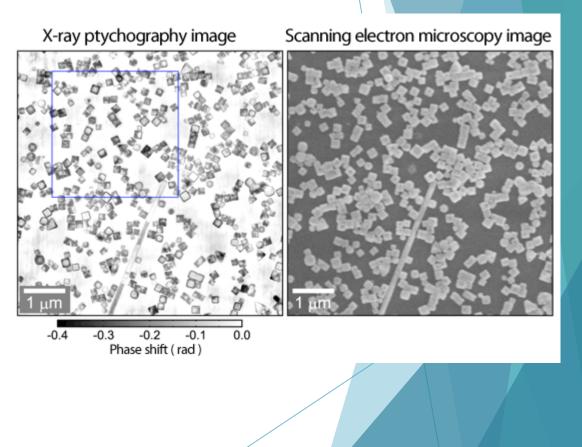
Drawbacks

- Expensive and complex equipment
- High vacuum is needed
- Sample preparation
- Slow: Time-consuming
- Poor statistics
- Artefacts



X-ray microscopy (XRM)

- Can provide spatial resolution imaging of a specimen in the aqueous state without the need for sample preparation, e.g. fixation, staining or sectioning
- The major limitation of conventional electron microscopes, such as transmission electron and scanning electron microscopes, is that they have to be operated under vacuum conditions.



Spectroscopic and related techniques

LIGHT SCATTERING METHODS

- static (SLS) also known as multi-angle (laser) light-scattering (MAL(L)S),
- dynamic light-scattering (DLS) or photon correlation spectroscopy (PCS)
- neutron scattering, small-angle neutron scattering (SANS).
- Nuclear magnetic resonance (NMR)



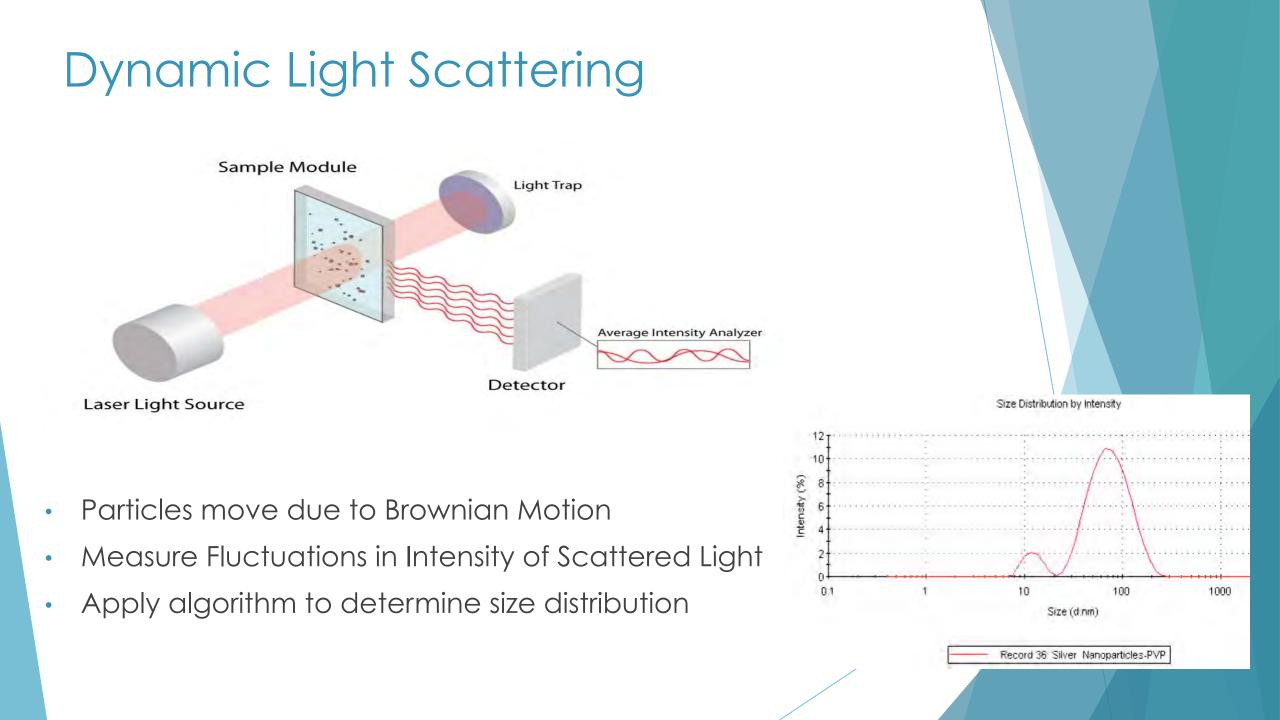
sizing nanoparticles state of aggregation in suspensions.



particle structure

particle shape

dynamics three-dimensional structure



Dynamic Light Scattering

Advantages

- Inexpensive
- Fast
- Good statistics
- No influence of beam
- Weighting for intensity, volume and number possible

Drawbacks

- Indirect measurement
- Only dispersions can be measured
- Influence of medium
- 'Spherical' particles
- No model for very elongated or irregular particles

Centrifugation and filtration techniques

Centrifugation and filtration techniques are well-established tools for the preparative, size fractionation of samples.

These are:

- low-cost
- high speed
- high volume



Ultracentrifugation (UC), for example, is a centrifuge system capable of very high spinning speeds for accelerations up to 1,000,000 g.

Chromatography and related techniques

Separation of nanoparticles in samples:

- rapid
- sensitive (detector-dependent)
- non-destructive



samples are available for further analysis.

By attaching traditional analytical tools (e.g. ICP–MS, DLS) as detectors to size separation techniques, it is not only possible to **quantify** different nanoparticles in food, water, biota and soil, but also to **characterise** or elementally **analyse** them.

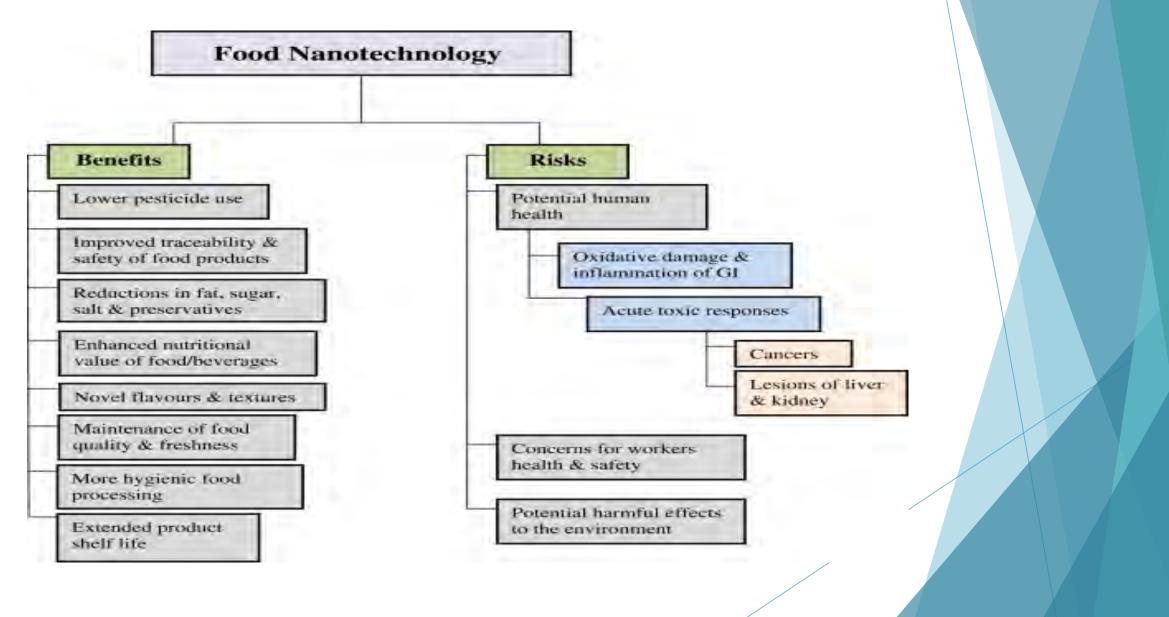
Size exclusion chromatography (SEC)

- A size exclusion column is packed with porous beads as the stationary phase.
- The pores of the column retain particles, depending on their size and shape.

Disadvantages

• Possible interactions of the solute with the solid.

Advantages/disadvantages



New EFSA guidelines on nanotechnology in food and feed. Risk assessment for human and animal health

The European Food Safety Authority (EFSA) has published the new guidelines on risk assessment and the effectiveness of nanosciences and nanotechnologies in the food chain. These are new foods, food contact materials, food and feed additives, pesticides.

- nano-silver
- nano-sensors

To be sure that nanotechnology in food does not harm health:

NMS must be evaluated in terms of safety,

the new risk assessment guidelines have been studied for **exposure** and for the **characterization of the hazards** of nanomaterials.



EFSA guidelines

- The use of a nanomaterial in food/feed will need to be assessed for safety to fulfil requirements of the relevant EU food laws, and in accordance with the provisions of this Guidance.
- The existing risk assessment paradigm for chemicals is also applicable to nanomaterials.
- The Guidance proposes a structured pathway for carrying out safety assessment of nanomaterial in food/feed and related applications, and provides practical suggestions for the types of testing needed and the methods that can be used for this purpose.
- The Guidance also highlights certain gaps where further research is needed to facilitate adequate safety assessment of materials that consist of small-sized particles.



Future perspectives

- The fate and potential toxicity of nanomaterials are not fully understood
- In addition to the benefits, nanotechnology can also assist in the detection of **pesticides**, **pathogens** and **toxins**, serving in the food quality tracking-tracing-monitoring chain.
- Furthermore, nanotechnology has the potential to transform our future food packaging materials, as part of an active and intelligent packaging system.

Conclusion



The associated health, safety, and environmental impacts should be addressed and regulated at the forefront.

To be successful in the long run, proper education of the public is also paramount in the introduction and development of nanotechnology in food system and there seems to be an urgent need to gather information on this subject.

THANKS FOR THE ATTENTION