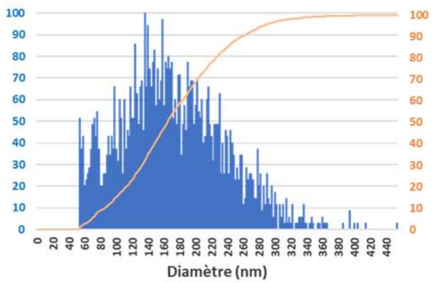
Workshop Nanoparticles : Analytical Strategies & Applications

Ultra Traces Analyses Aquitaine

SCL

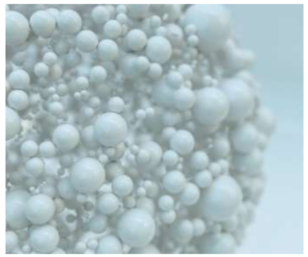
Adera

spICP-MS & inorganic nanoparticles : implementation, optimization, validation and application to consumer products

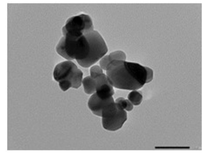


Mathieu Menta

mathieu.menta@univ-pau.fr



NANO < 100nm



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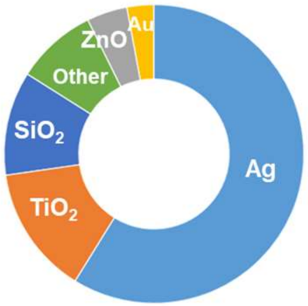
General information on inorganic nanoparticles

A booming market, an increasing use in different fields:



Particles	Products	Main use	Food Additive
TiO_2	Food (sweets & sauces), paints, hygiene products, food packaging, cosmetics, drugs …	✓White pigment ✓UV filter (in combination with ZnO) ✓Flavour enhancer (dry fruits, soups, mustard …)	*E171*
Ag	Food packaging, textiles, food, food supplements, hygiene products, medical devices	✓Antimicrobial agent ✓Decorative agent for patisserie …	*E174*
SiO_2	Food, powder soups, coffee, hygiene products, mayonnaise …	✓Anti-caking agent ✓Improvement of texture and smoothness …	*E551*
Iron oxide	Food	✓Colour agent ✓Increase of bioavailability	*E172*

A large media coverage


Des nanoparticules cachées dans nos assiettes

100 % des bonbons et gâteaux que nous avons testés contiennent du dioxyde de titane sous forme « nano ». Contrairement aux dires des fabricants.

Des traces de nanomatériaux détectées dans du lait maternisé

Par Lucile Morin — 29 mai 2016 à 21:11

Libération

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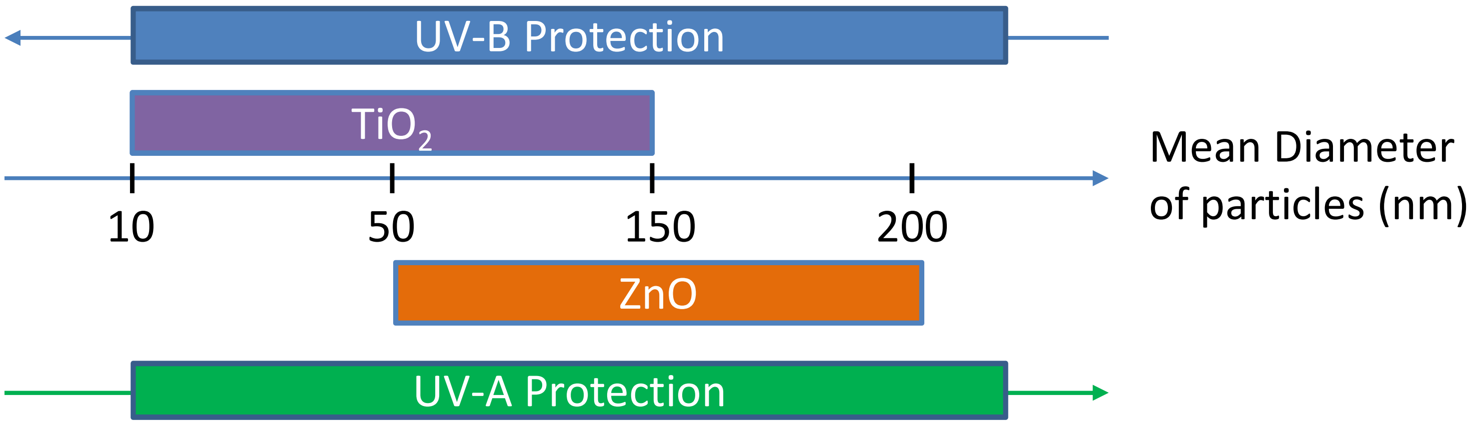
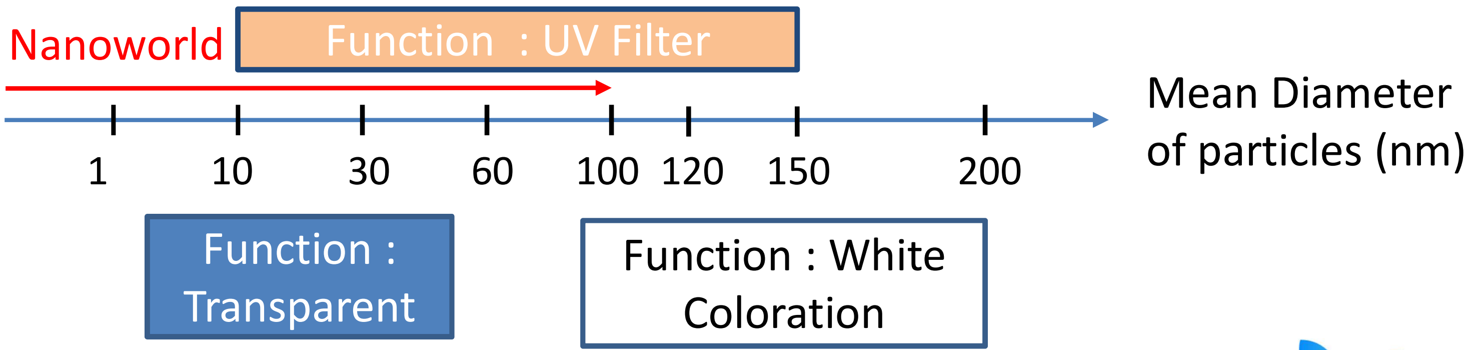
Inorganic nanoparticles in Cosmetics

TiO2 & ZnO in Sunscreens : Mineral filters, inert and opaque powders, used for light reflection



Example of TiO_2



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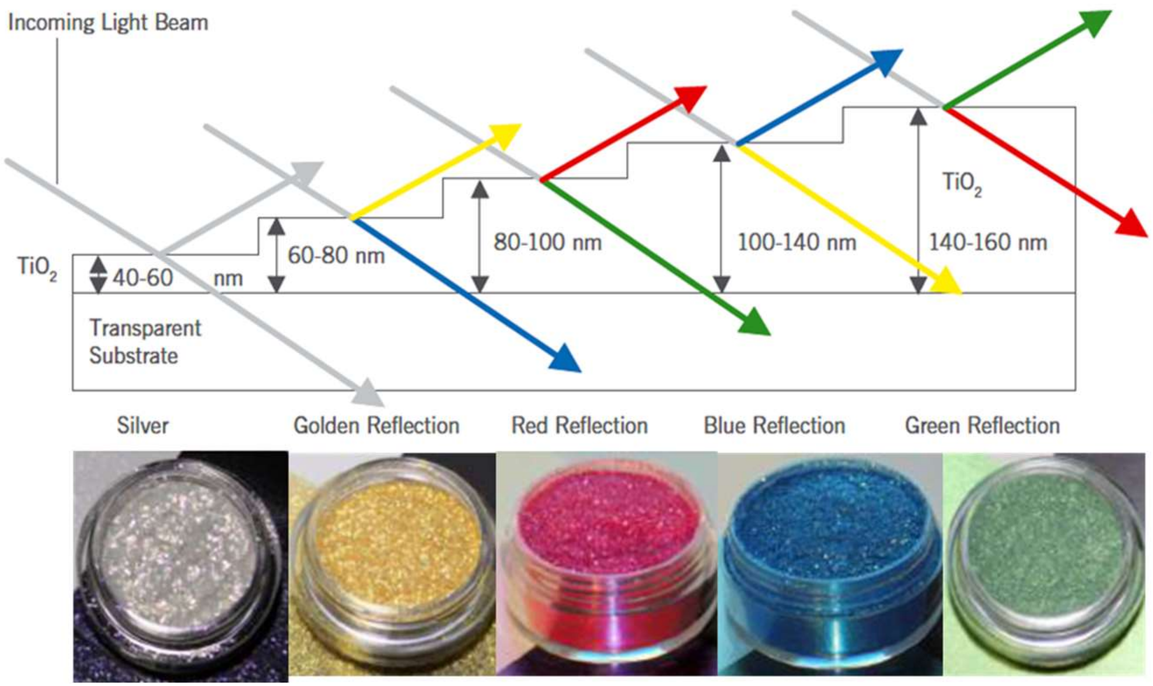
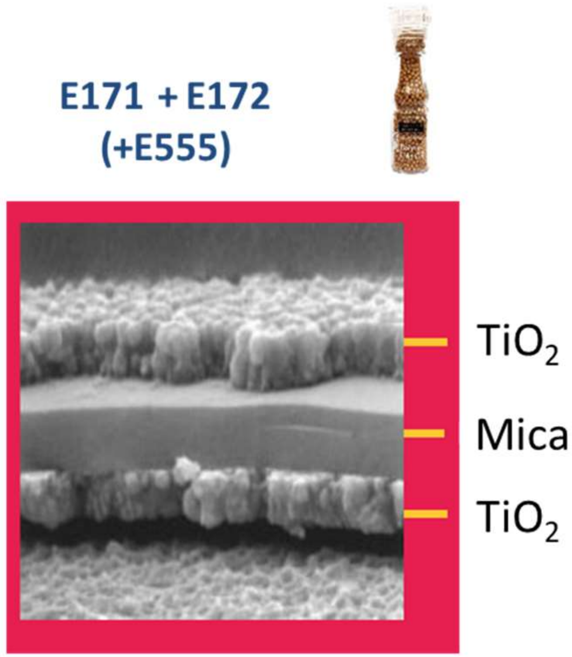
Inorganic nanoparticles & Food Technology

Conventional use of inorganic food additives: obtain new properties at the nanoscale

For example : glittery/shiny effect in confectionery, spices, in gourmet cuisine, etc.





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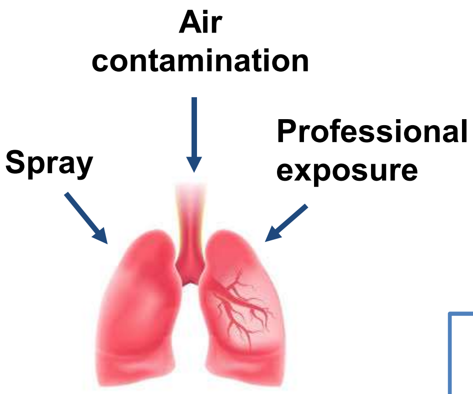
TiO2 NPs: health effects

HEALTH EFFECTS OF TiO_2 NPs

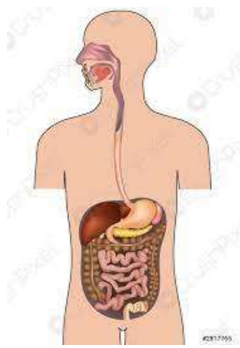
Inhalation

Possible carcinogen (group 2B) *(from IARC)*



Since 2020, in food, the use of E171 is bannished in France and since 2021 in Europe

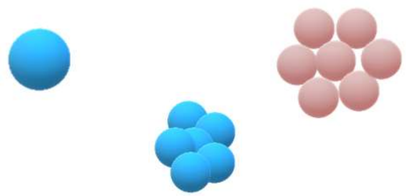
Ingestion



Daily intake :

- **0.03** mg per kg body weight (adult)
- until **13** mg per kg body weight (children < 10 years)

Possible carcinogen by ingestion ?



A relatively vague regulatory framework…

How to differentiate them ?

Particle : minute piece of matter with defined physical boundaries;
Agglomerate : collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components;
Aggregate : particle comprising of strongly bound or fused particles

European Commission (Recommendation 2011/696/EU -18/11/2011)

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.
In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %

Which ones?

Which parameter to measure?
Which measurand?

Few techniques able to detect 1 nm

Other definitions :

ISO (*Norm ISO TS 80004-1)*
- Material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale.(Nanometric scale = 1 to 100 nm)

Cosmetic *Directive n°1223/2009*
- Insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm
- The name of the nanomaterials must appear in the ingredient list

Other regulations :
- **Biocide** (CE n°528/2013)
- **Additives** (CE n°1333/2008)
- **INCO** (CE n°1169/2011)
- etc.

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Regulation and labelling

Regulation 1169/2011 (European Parliament): provides for labelling of NMs used as ingredients

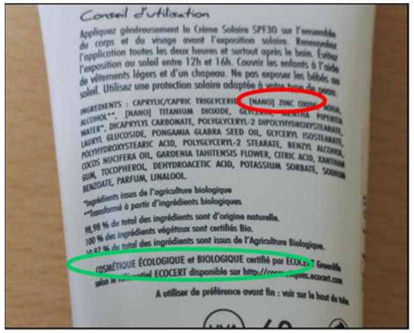
Presence of nanomaterials subject to a marking obligation for cosmetics, biocidal products and foodstuffs.

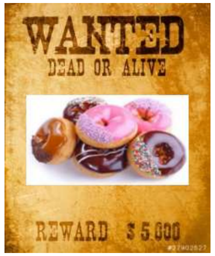
Target audience: Control Authority, Formulators/Distributors of Finished Products, Manufacturers of Raw Materials, etc.: Is the product properly labelled (RCE No. 1881/2006)?

- Presence of particles?
- Particle composition?
- Nano particles?
- What distribution?

→ *Development of an analytical strategy for the size characterization of additives in food and cosmetic products*

→ *Need for appropriate analytical techniques for regulatory purposes: accuracy, speed, cost of analysis*





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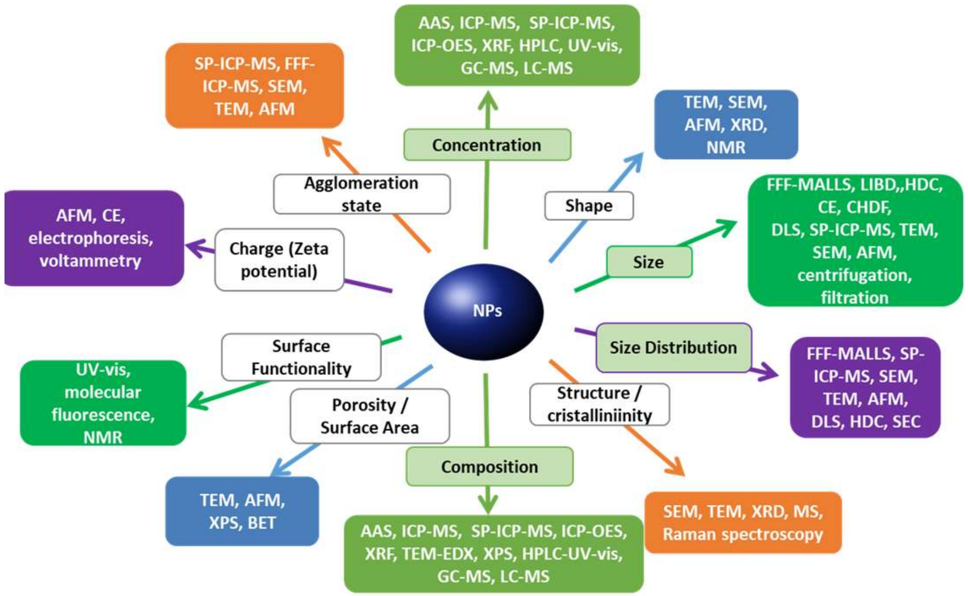
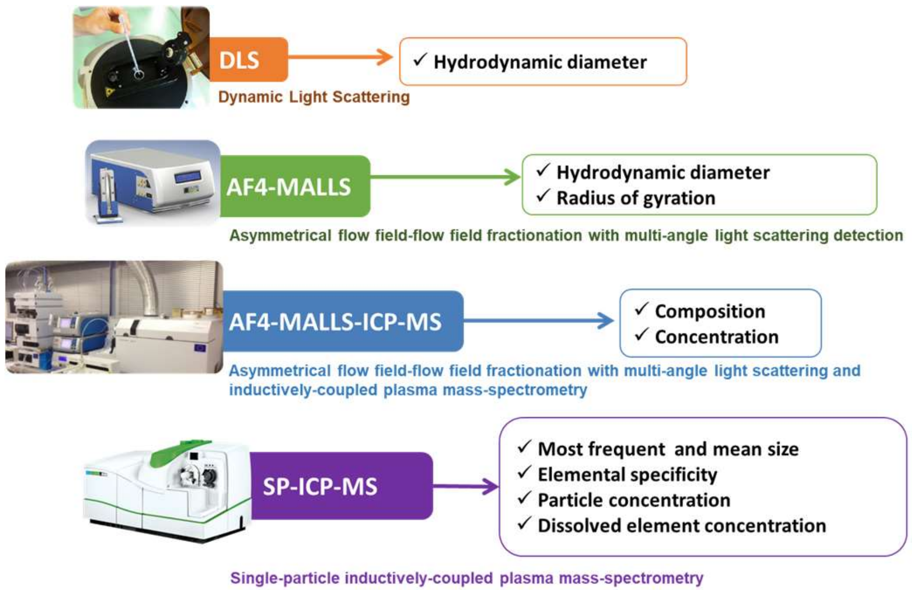
What analytical strategy should be implemented?

An analytical arsenal at your disposal



Available techniques in UT2A facilities



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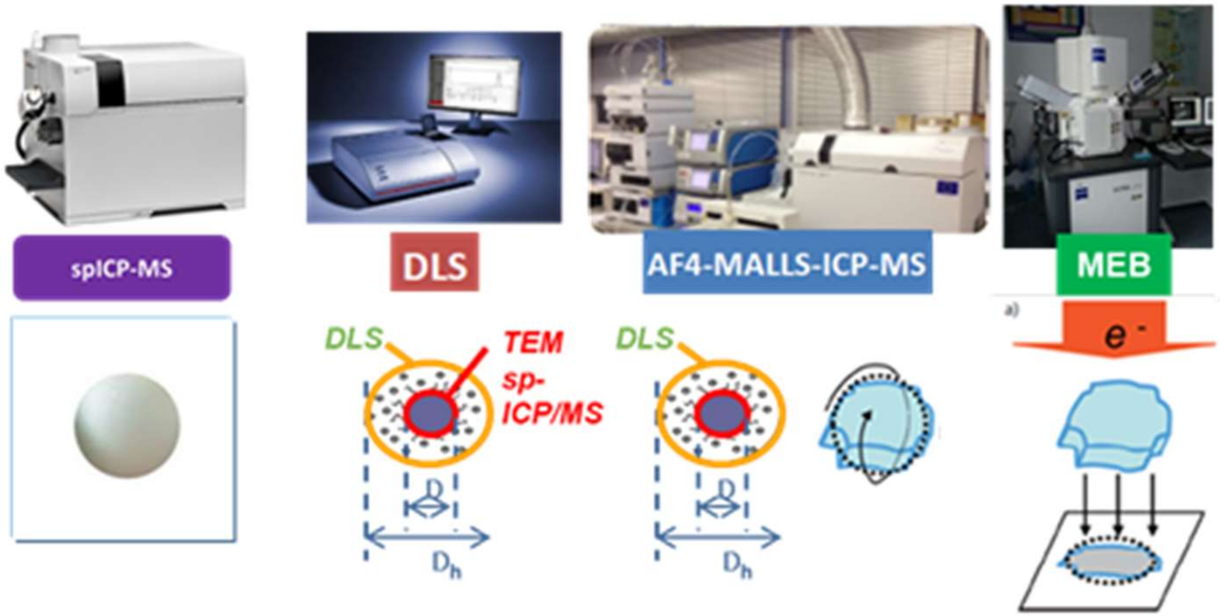
What analytical strategy should be implemented?

Pay attention to the parameter / measurand according to the technique used +

Lack of a standardized or recognized characterization method & CRM



spherical equivalent diameter

Hydrodynamic diameter

Giration diameter

projected geometric diameter

An essential multi-technical approach

Cross-referencing information obtained by different techniques in order to develop robust and reliable analytical strategies

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Nanoparticles characterization by Single Particle mode (spICP-MS)

The "individual counting" of particle by spICP-MS is a technique:

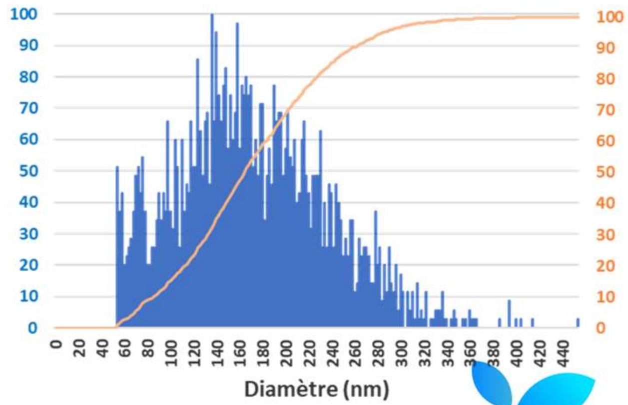
- recent, booming, adapted to the analysis of metallic nanomaterials
- provide answers in terms of composition, quantity, and size distribution of metal nanoparticles suspended in aqueous matrices.

simple
fast
sensitive
specific

Agilent Technologies

Supposedly "spherical" shape
Minimum variable diameter depending on the elements, samples and matrices

Information given:
- Average / median / modal diameter
- Particle number distribution
- Particulate concentration
- Dissolved element content



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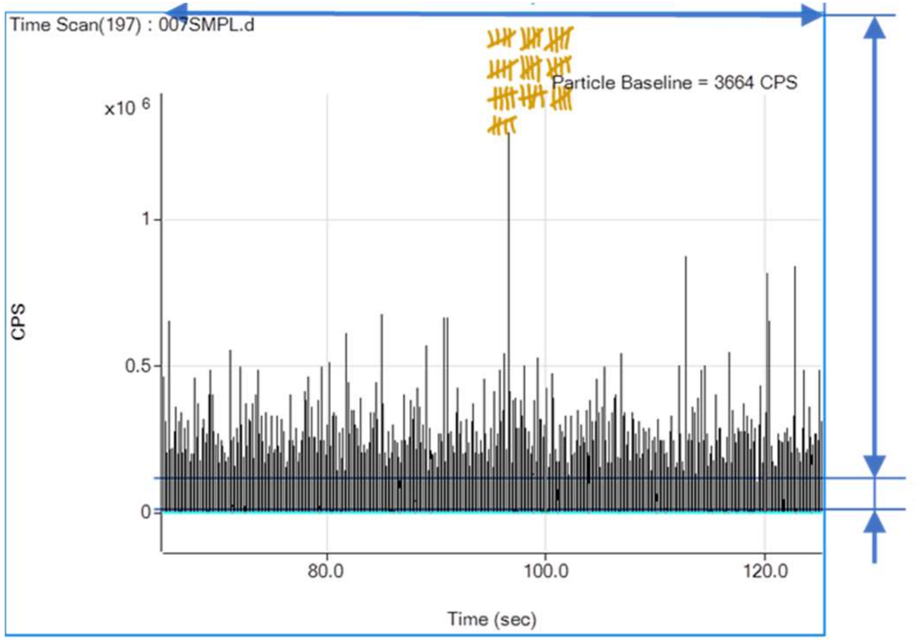
What can be observed in a few tens of seconds by spICP-MS ?

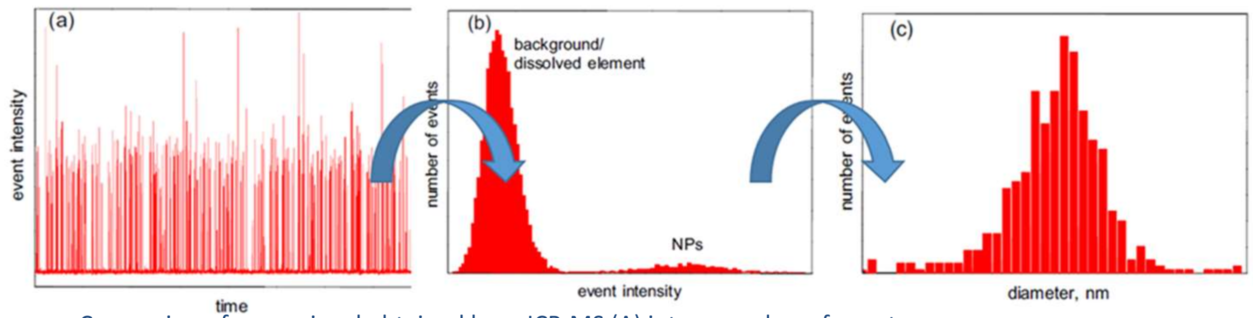
Raw data spICP-MS

Number of particles
Particulate mass
Ionic concentration



Size distribution of particles

Conversion of raw data according to successive calculations
Automated in the latest generation of software and devices
Ability to create custom calculation macros



Conversion of a raw signal obtained by spICP-MS (A) into a number of events as a function of the signal strength (B) and then as a function of the particle size (C).

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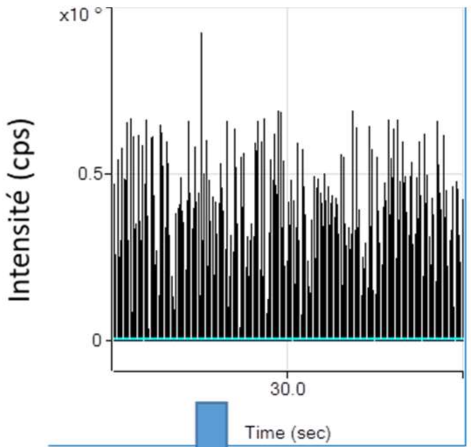
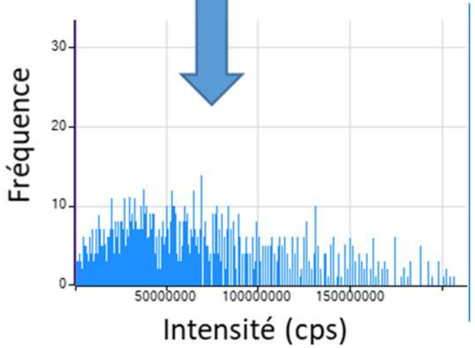
Data processing in spICP-MS

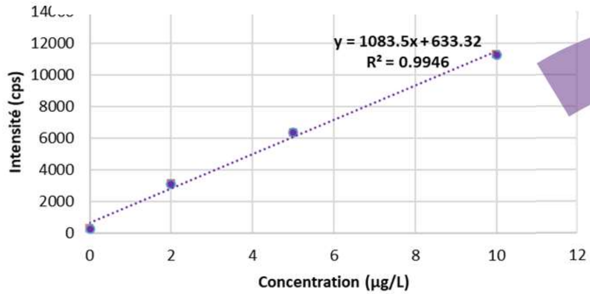


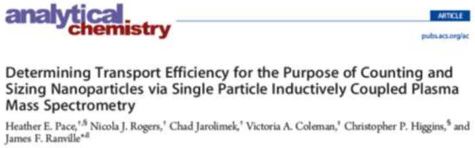


$$N_P = \frac{f(I_P)}{q_{liq} * \eta_n}$$

NPs Particulate concentration (particles/volume)

Ionic calibration

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ARTICLE

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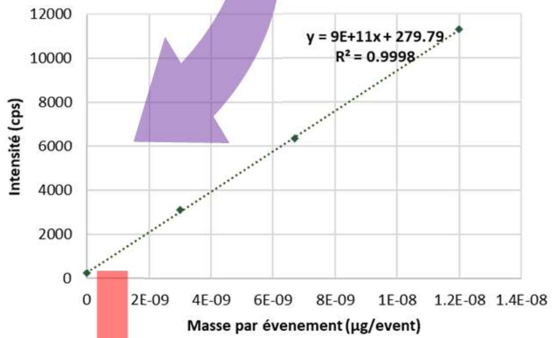
Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry

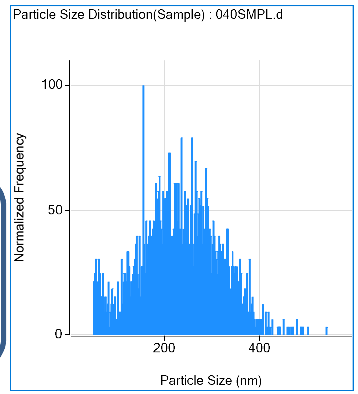
Heather E. Pace, Nicola J. Rogers, Chad Jarolimek, Victoria A. Coleman, Christopher P. Higgins, and James F. Ranville

$$W = [\eta_n * q_{liq} * t_{dt} * C]$$

$$m_p = f_a^{-1} * \left[\frac{((I_P - I_{Bgd}) * \eta_i) - b}{m}\right]$$



$$d = \sqrt[3]{\left[\frac{6 * m_P}{\rho * \pi}\right]}$$



Size distribution in number of particles

Importance of the transport efficiency

In spICP-MS, as well as in ICP-MS in general, when conventional sample introduction systems are used, only a fraction of the nebulized suspension effectively reaches the plasma (1–15%).

The precise determination of this fraction, which is defined as transport efficiency (η_n)is fundamental for the correct determination of both particle number size distribution and concentration

3 main calculation methods

	method 1	method 2	method 3
replicate	waste collection (%)	particle size (%)	particle frequency (%)
day 1 (12/1/10)	[a]	9.1 ± 0.2	9.0 ± 0.9
day 2 (12/22/10)	14.4 ± 1.2	8.8 ± 0.4	8.4 ± 1.1
day 3 (1/12/11)	14.5 ± 0.7	8.6 ± 0.2	8.7 ± 0.7

[a] Measurement not reported due to sampling error.

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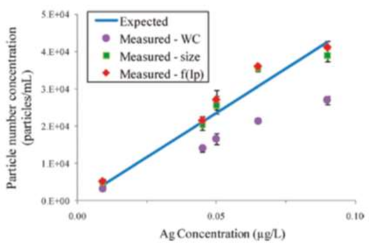
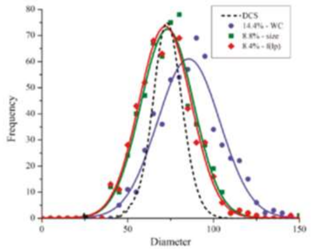
Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry

Heather E. Pace,[†,§] Nicola J. Rogers,[†] Chad Jarolimek,[†] Victoria A. Coleman,[‡] Christopher P. Higgins,[§] and James F. Ranville[*,∥]

Method 1 "Waste collection" gives the best efficiencies

Methods based on size (2) or frequency (3) give similar results





Method 1: Problem of underestimation of particulate concentration and overestimation of size

Methods 2 and 3: Results in accordance with theoretical values

Method 2 is the most used

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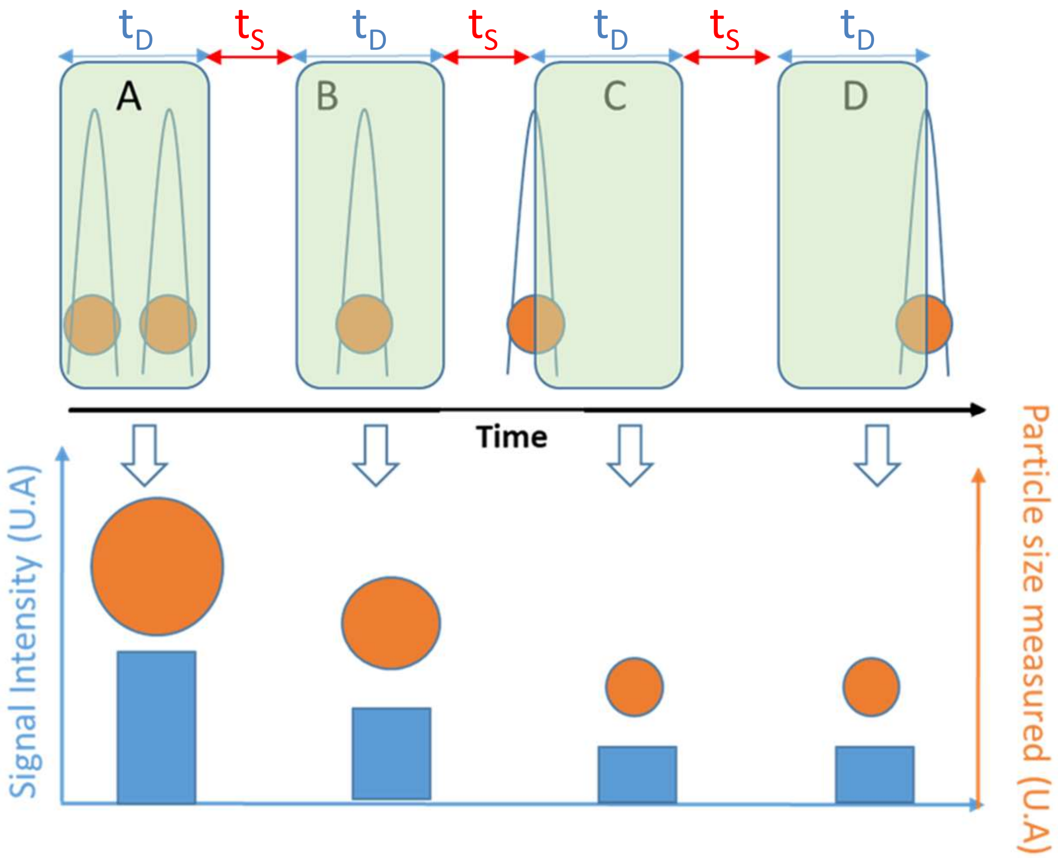
Measurement of a random event with a regular interval (Dwell time)

Conventional principle of signal acquisition by ICPMS



- Dwell time (t_D)= Acquisition time
- Settling time (t_S)=Stabilization time between two measurements

☹ **Inappropriate counting**
Wrong particle size

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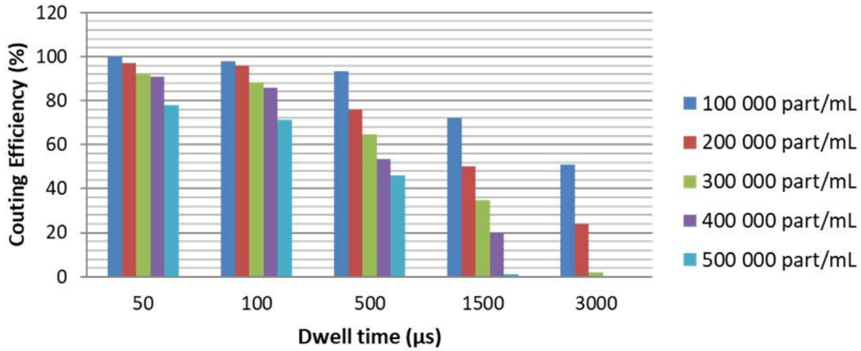
Influence of the Dwell time

Effect of Dwell Time on NPs quantification

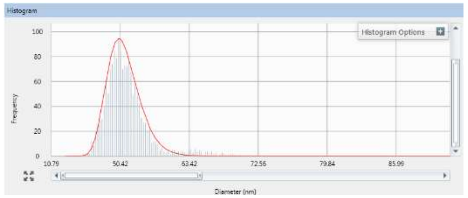
200 000 part/mL dwell time (50 µs)

200 000 part/mL dwell time (3000 µs)

Gold NPs 50 nm

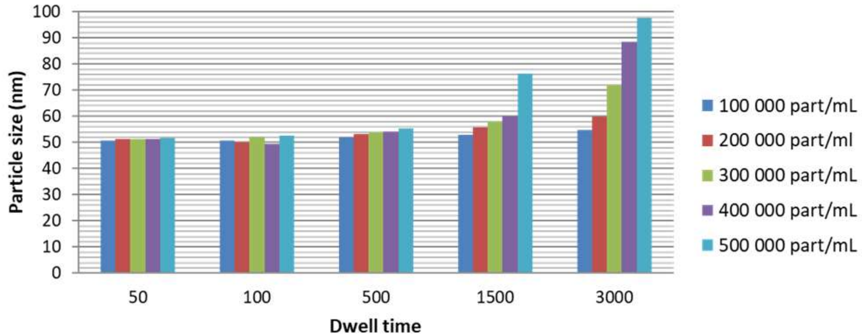


Effect of Dwell Time on NPs size measurement



300 000 part/mL dwell time (100 µs)

300 000 part/mL dwell time (1500 µs)



PerkinElmer
For the Better

☹ **Inappropriate counting**
Wrong particle size

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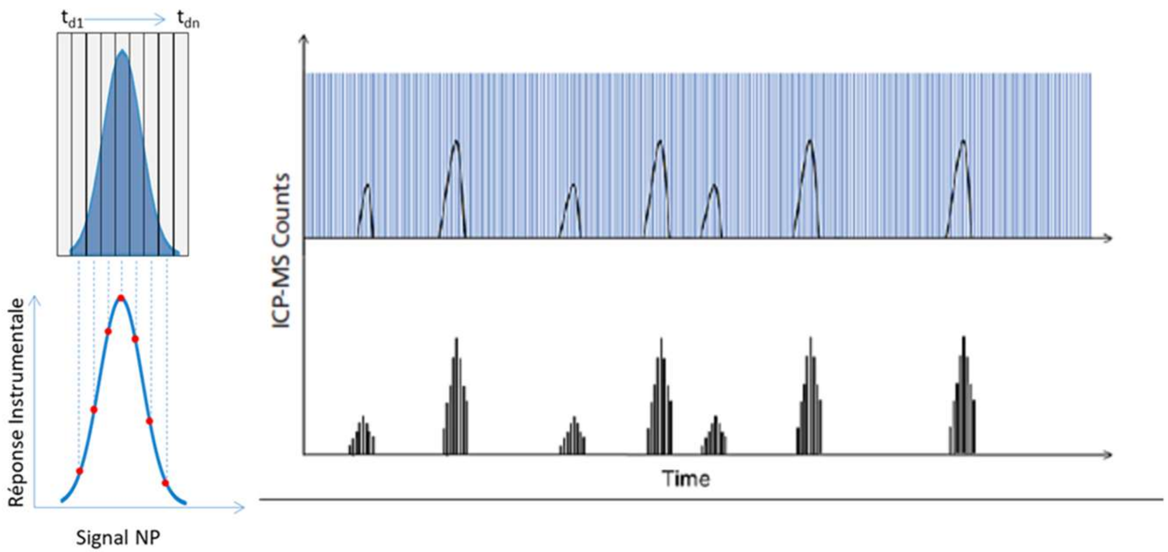
Influence of the Dwell time



☺ **Adapted counting**
Good particle size

- **No settling time for the analysis of a single mass**.
- Continuous rapid acquisition
- Dwell Time reduced to 0.05 to 10 ms

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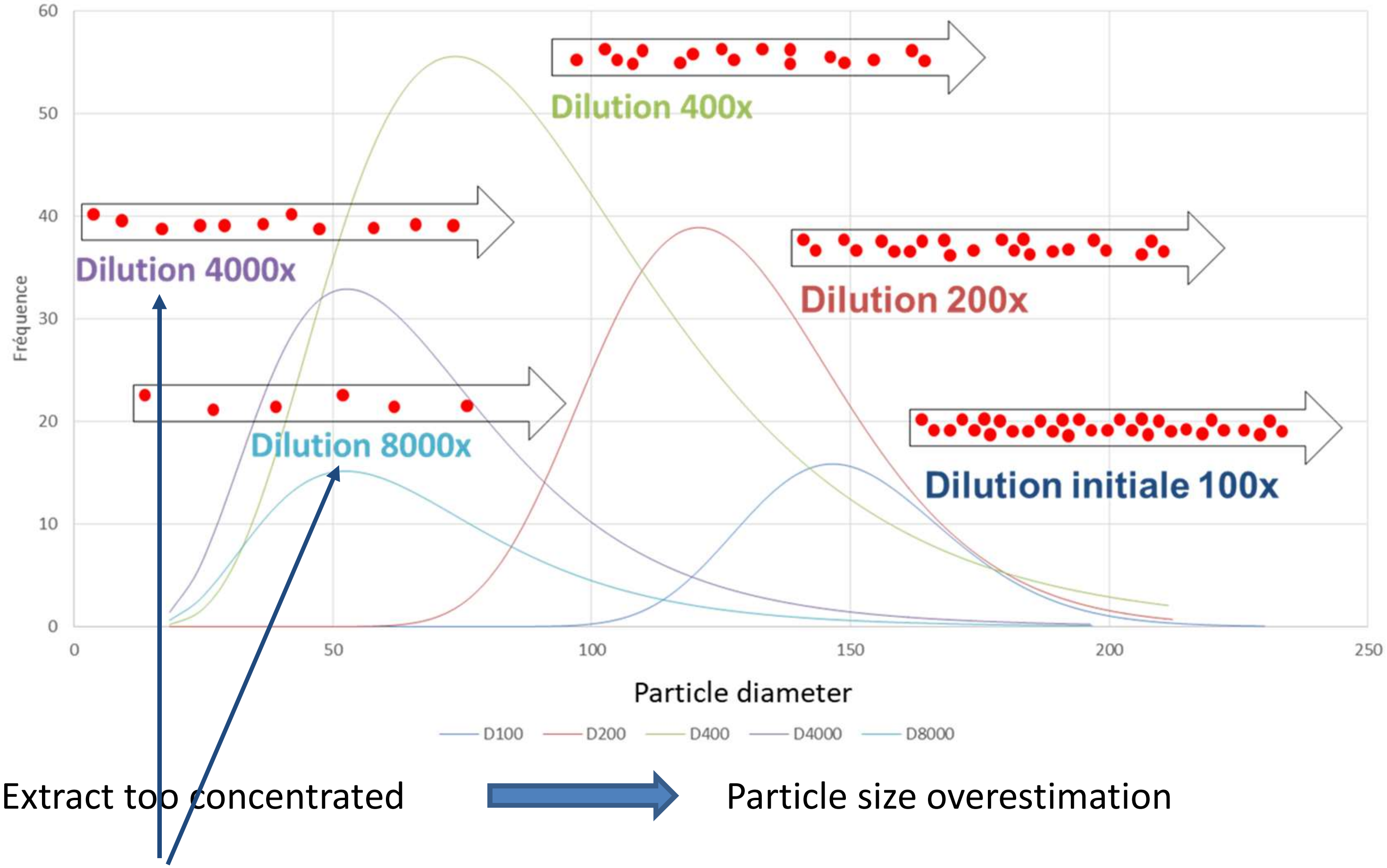
Influence of the sample extract/suspension dilution factor



Extract too concentrated → Particle size overestimation

Conditions & results selected: same granulometric properties and quantification of particles in accordance with the dilution factors

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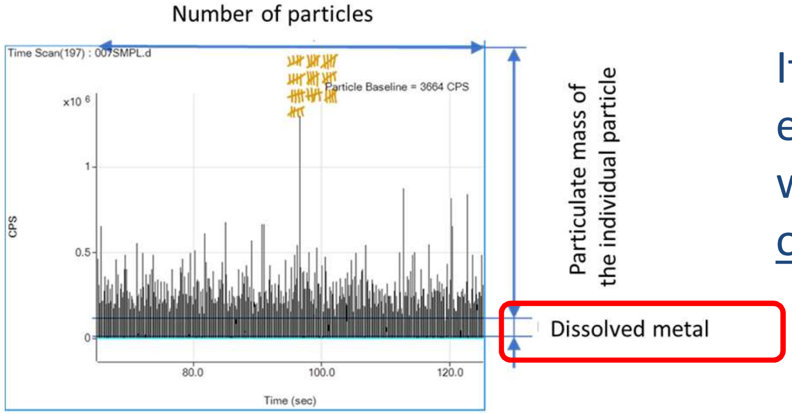
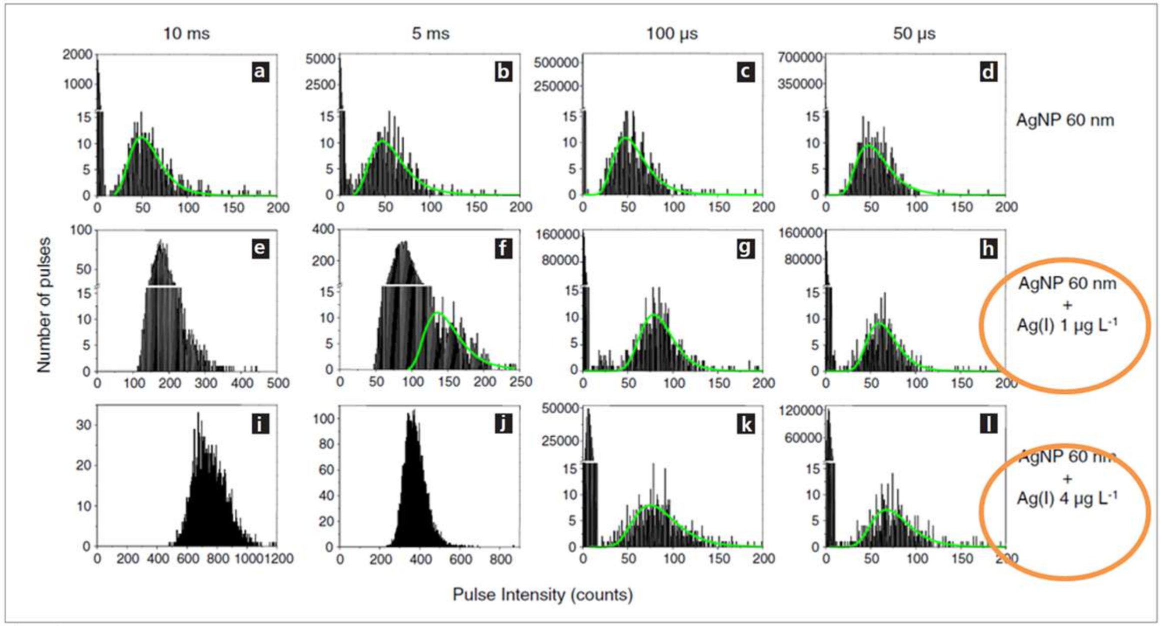
Influence of the ionic backgound



It is the distinction between the particles events from analytical background noise that will make the difference! <u>With the incidence of dwell-time!</u>



Once all these parameters checked with ionic and or nanoparticles standards, it's time for analysis with real samples !

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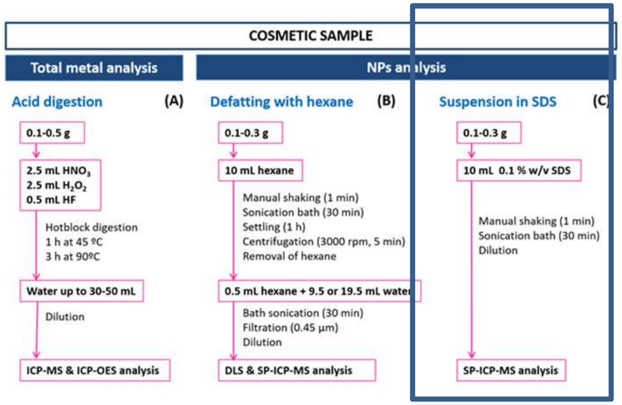
spICP-MS: tool of choice for the screening of inorganic nanoparticles?

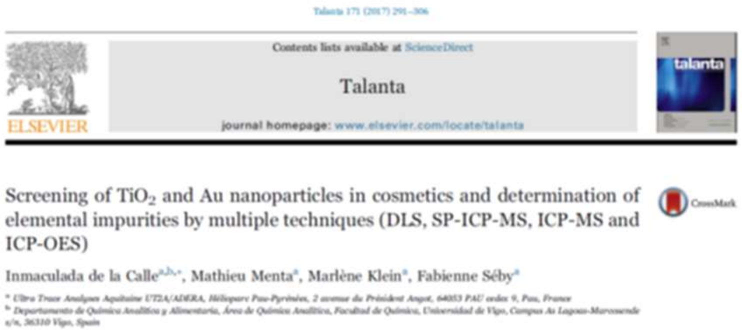
Objectives:

Propose a fast and efficient screening method for the detection of inorganic NPs in consumer products

Discriminate products subject to regulation : Presence of nanoparticles ? YES or NO ?

Do not accurately characterize the particle size distribution or quantify them





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Screening of TiO_2 and Au nanoparticles in cosmetics and determination of elemental impurities by multiple techniques (DLS, SP-ICP-MS, ICP-MS and ICP-OES)

Inmaculada de la Calle[a,b,*], Mathieu Menta[a], Marlène Klein[a], Fabienne Séby[a]

[a] Ultra Trace Analyses Aquitaine UT2A/ADERA, Hélioparc Pau-Pyrénées, 2 avenue du Président Angot, 64053 PAU cedex 9, Pau, France
[b] Departamento de Química Analítica y Alimentaria, Área de Química Analítica, Facultad de Química, Universidad de Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain

Sample preparation as simple and fast as possible

Tests carried out on 16 cosmetic samples:

Code	Type of sample	Characteristcs (label, metals and other compounds)	Price (€)
1	Anti-dandruff shampoo A	Zn-pyrithione-menthol, dimethicone	4
2	Anti-dandruff shampoo B	Zn-pyrithione, $ZnCO_3$, $MgCl_2$, dimethicone	4
3	Shampoo & hair conditioner	$MgCl_2$, KCl, $AlCl_3$, $CuSO_4$, $MgCl_2$, $MnCl_2$, $ZnCl_2$, dimethicone	2
4	Sunscreen SPF 50 (spray)	Titanium dioxide (nano), trimethoxycaprylysilane	15
5	Sunscreen SPF 30 (cream)	Titanium dioxide (nano)/titanium dioxide, iron oxides, dimethicone, aluminum hydroxide	15
6	Antiwrinkle day cream	Titanium dioxide (nano), silica, dimethicone, sodium acrylate/sodium acryloydimethyl taurate copolymer	3
7	Day cream SPF 10 with gold	Colloidal gold, synthetic peptide	20
8	Facial serum A	Golden microseaweed 0* with particles of gold, titanium dioxide, mica	5
9	Day cream SPF 10 A	Golden microseaweed 0* with particles of gold, titanium dioxide, mica	5
10	Night cream A	Golden microseaweed 0* with particles of gold	5
11	Facial serum B	Titanium dioxide, mica, gold, cyclopentasilonxane, divinyldimethicone dimethicone copolymer, PEG/PPG/14/4 dimethicone	15
12	Day cream SPF 10 B	Titanium dioxide, mica, iron oxides, gold, cyclopentasilonxane	15
13	Night cream B	Titanium dioxide, mica, iron oxides, gold, cyclopentasilonxane, dimethicone	15
14	Toothpaste for sensitive teeth	Titanium dioxide, KNO_3, NaF_2, silica	5
15	Toothpaste	Titanium dioxide, hydrated silica, mica, phtalocyanine blue BN of copper (cupferphtalocyanine), trisodium phosphate	2
16	Lip balm SPF 40	Titanum dioxide (nano), alumina, perfume (Fragance), silica (nano)	5

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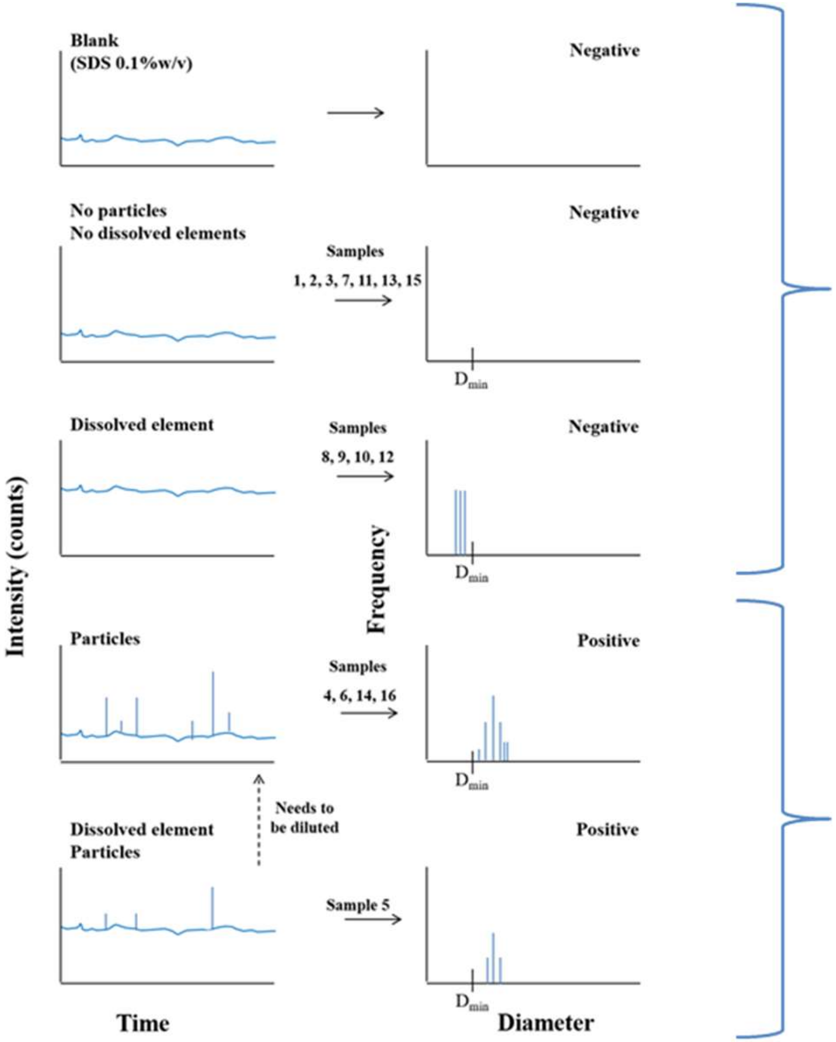
Screening of nanoparticles in cosmetic samples : Presence or Absence ?



Negative Screening: Absence of nanoparticles

Positive Screening: Presence of nanoparticles

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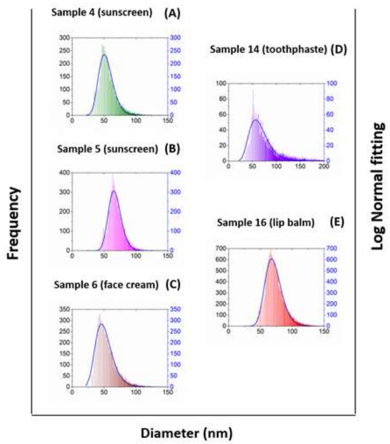
Screening of nanoparticles in cosmetic samples : Presence or Absence ?

TiO_2 NPs screening by SP-ICP-MS.

Code	Type of sample	Label: TiO_2	Label: TiO_2 'nano'	SP-ICP-MS screening of the presence of TiO_2 NPs
1	Shampoo	No	No	No
2	Shampoo	No	No	No
3	Shampoo & hair conditioner	No	No	No
4	Sunscreen SPF 50 (spray)	Yes	Yes	Yes
5	Sunscreen SPF 30 (cream)	Yes	Yes	Yes
6	Anti-wrinkle day cream	Yes	Yes	Yes
7	Day cream with SPF 10	No	No	No
8	Facial serum	Yes	No	No
9	Day cream SPF 10	Yes	No	No
10	Night cream	No	No	No
11	Facial serum	Yes	No	No
12	Day cream SPF 10	Yes	No	No
13	Night cream	Yes	No	No
14	Toothpaste (for sensitive teeth)	Yes	No	Yes
15	Toothpaste	Yes	No	No
16	Lip balm protector SPF 40	Yes	Yes	Yes

Only samples including the [Nano] label show positive screening... except one (14)



Since this product is labeled [Nano]

From a regulatory point of view, the spICP-MS already answers the first question relating to labelling: Presence of particles, YES or NO?

Products with positive screening : Deeper characterization required (Particle size distribution & quantification)

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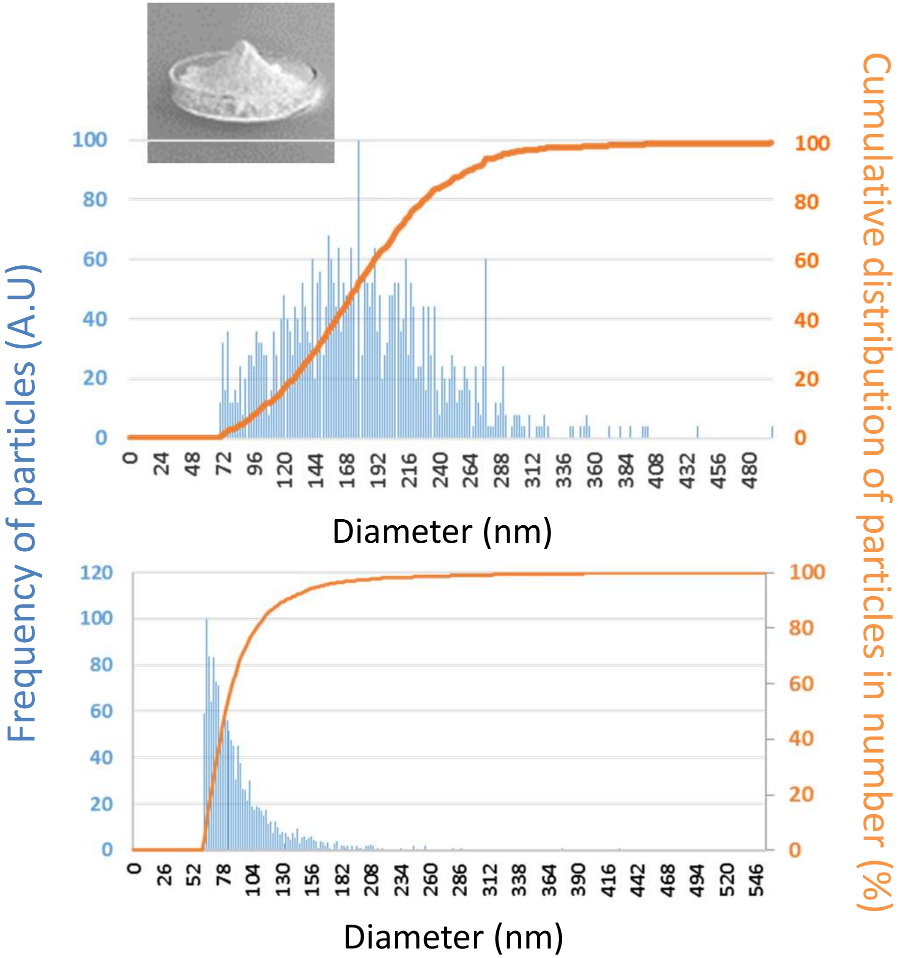
UNTWIN

Characterization of TiO2 NPs in food & cosmetic additives



Parameter	Results
% of particles < 100 nm	11 ± 2
Mean Diameter(nm)	188 ± 6
Median Diameter (nm)	176 ± 3
$[TiO_2]_{particulate}$ (g/kg)	259 ± 6

Parameter	Results
% of particles < 100 nm	69 ± 4
Mean Diameter(nm)	93 ± 2
Median Diameter (nm)	82 ± 3
$[TiO_2]_{particulate}$ (g/kg)	846 ± 12

According to INCO or COSMETIC Regulation the both products are "nanos"
According to Def. EU, only the cosmetic additive is...

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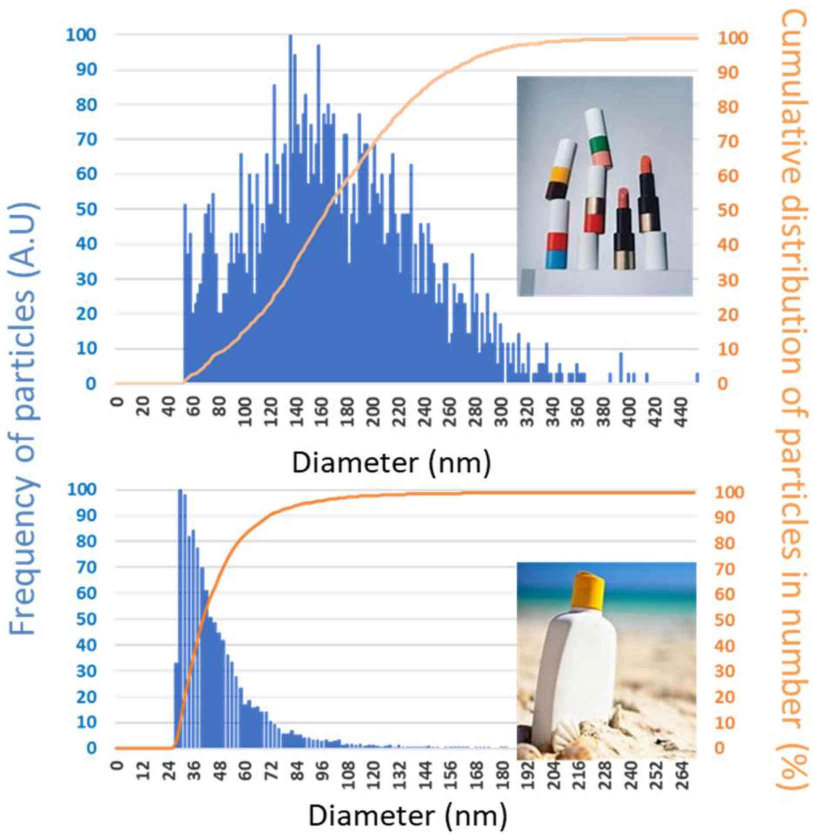
FUNTOMIN

Characterization of TiO2 NPs in cosmetic products



Parameter	Résults
% of particules < 100 nm	16 ± 2
Mean Diameter(nm)	173 ± 3
Median Diameter(nm)	161 ± 6
$[TiO_2]_{particulate}$ (g/kg)	1.35 ± 0.03

Parameter	Résults
% of particules < 100 nm	97.1 ± 0.5
Mean Diameter(nm)	46 ± 2
Median Diameter(nm)	41 ± 4
$[TiO_2]_{particulate}$ (g/kg)	241 ± 13

According to COSMETIC Regulation the both products are "nanos"
According to Def. EU, only the latest cosmetic additive is...

From a regulatory point of view, the spICP-MS meets the expectations of manufacturers and control laboratories... **but are the results reliable**?

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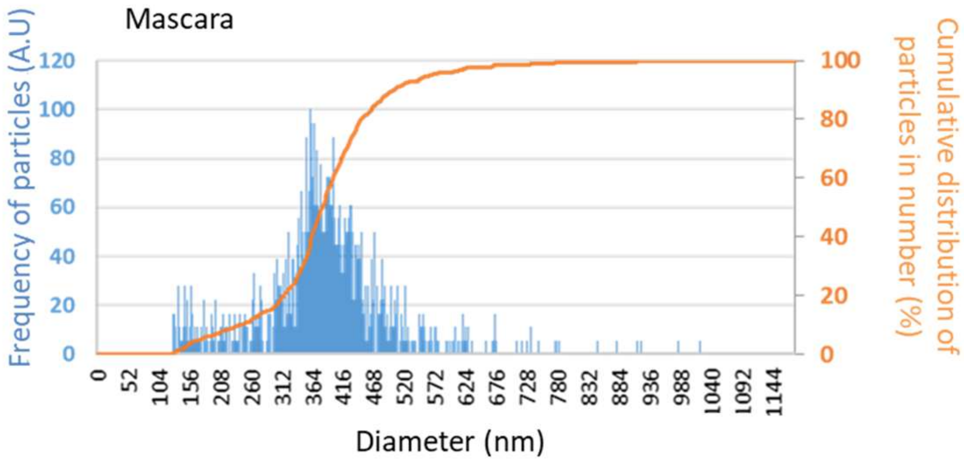
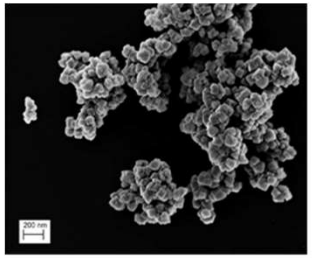
Characterization of Iron Oxydes in cosmetic products

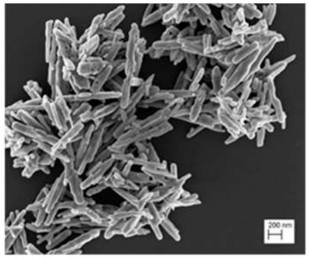


Parameter	Résults
% of particules < 100 nm	0
Mean Diameter(nm)	386 ± 7
Median Diameter(nm)	382 ± 3
$[Fe]_{particulate}$ (g/kg)	26 ± 3

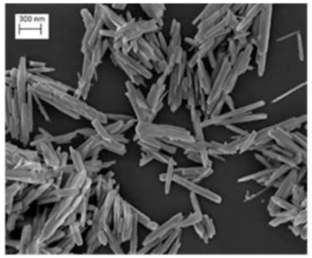
SCL



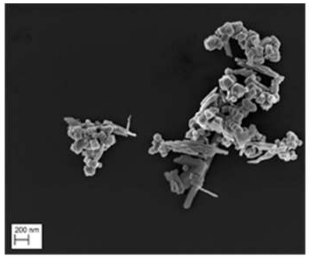
Red iron oxide
E172ii (Fe_2O_3)
spheroids



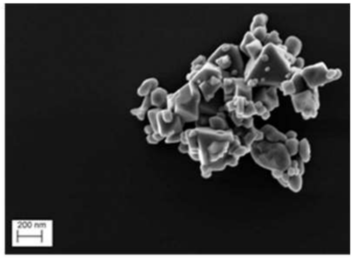
Red iron oxide
E172ii (Fe_2O_3)
needles



Yellow iron oxide
E172iii
($FeO(OH).H_2O$)
needles



Brown iron oxide
E172i (Fe_3O_4)
+ E172ii (Fe_2O_3)
needles + spheroids



Brown iron oxide
E172i (Fe_3O_4)
+ E172ii (Fe_2O_3)
pyramids + spheroids

Many mixtures and morphologies on the market
Significant differences between spICP-MS & SEM results (97 % of particles < 100 nm)

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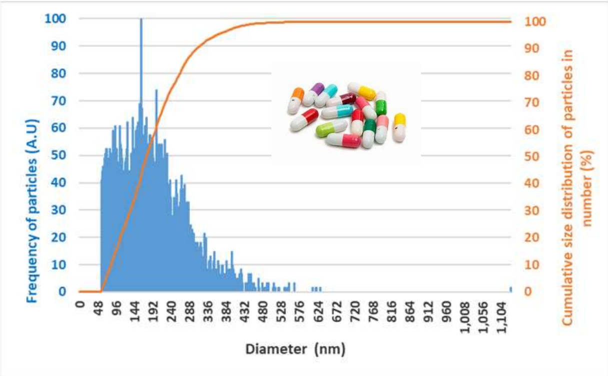
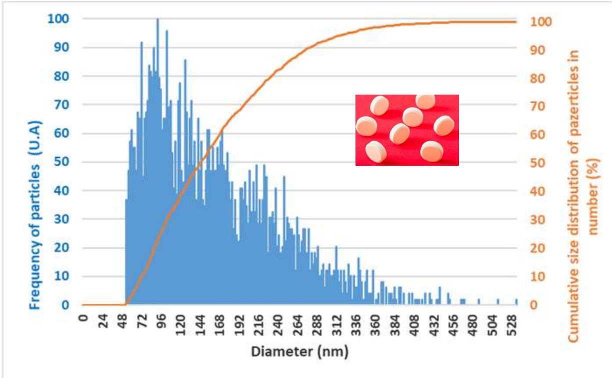
UNTWIN

Characterization of TiO2 NPs in pharmaceuticals products

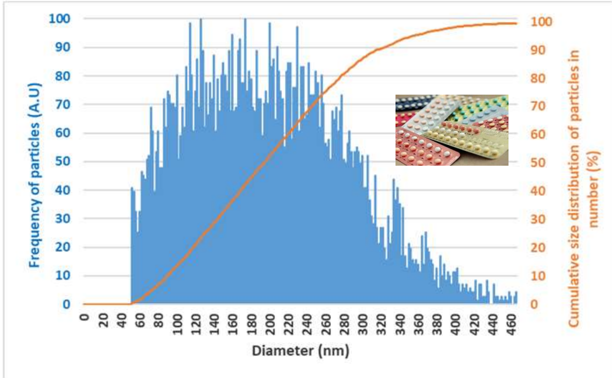
Drug Capsule



Compressed Drug



Contraceptive Pill



Sample	Parameter			
	% < 100 nm	Mean Diameter (nm)	Median Diameter (nm)	[TiO2 NPs] g/kg
Drug capsule	16 ± 3	192 ± 4	176 ± 6	6.0 ± 0.3
Compressed Drug	25 ± 7	165 ± 7	147 ± 8	0.49 ± 0.02
Contraceptive pill	14 ± 1	203 ± 4	195 ± 8	3.2 ± 0.2

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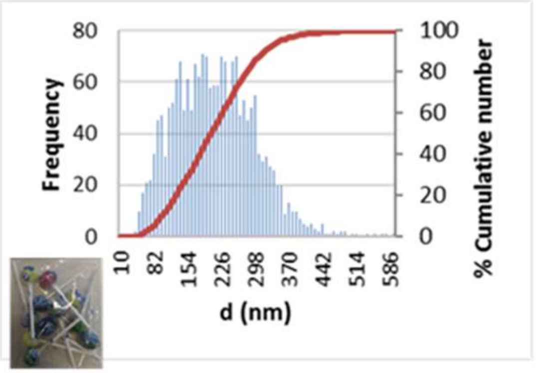
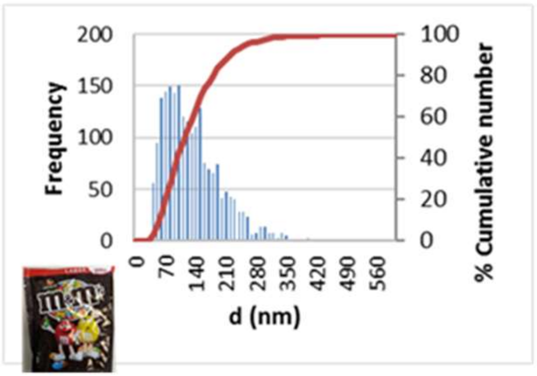
The reliability of the results obtained by spICP-MS?
Legitimate question

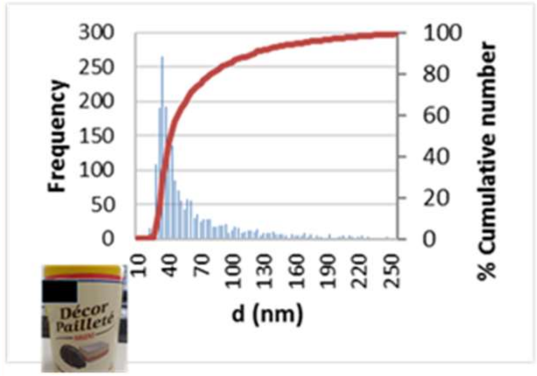
Characterization of TiO2 particles in food products



Mean (nm)	Median (nm)	% < 100 nm	D10 (nm)
212	208	10%	100



Mean (nm)	Median (nm)	% < 100 nm	D10 (nm)
133	118	38%	59



Mean (nm)	Median (nm)	% < 100 nm	D10 (nm)
61	42	86%	31

SCL

- Wide variety of samples, wide variety of size distributions
- Lack of a standardized or recognized characterization method
- Absence of CRM (certified reference material) in real matrices containing nanoparticles characterized by different methods

Need for rigorous analytical strategies to ensure the relevance of the results obtained and method validation

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UNTWIN

Criteria for analytical validation of operating conditions in spICP-MS

Implementation of an essential self-control strategy

- Performance evaluation:
  - instrumental performance: cf. manufacturer procedures, linearity of the response for the ionic elements analyzed, accuracy of the measured sizes for nanoparticles standards and concentrations measured for nanoparticles standards (if certified in concentration); Determine parameters such as: LOD_{NP} (particles/L), LOD_{MP} (ng/L), LOD_{S} (nm), etc.

- Acceptance Criteria
  - number of events, maximum ionic concentration, etc., for risk mitigation (co-event, bad size estimation, contamination)
  - Obtain identical results for several different dilution factors while respecting the previous criteria

Propose tools, such as a spreadsheet, to decide on the reliability of the analytical method
XP ISO/TS 19590 standard can serve as the basis for establishing this spreadsheet / validation

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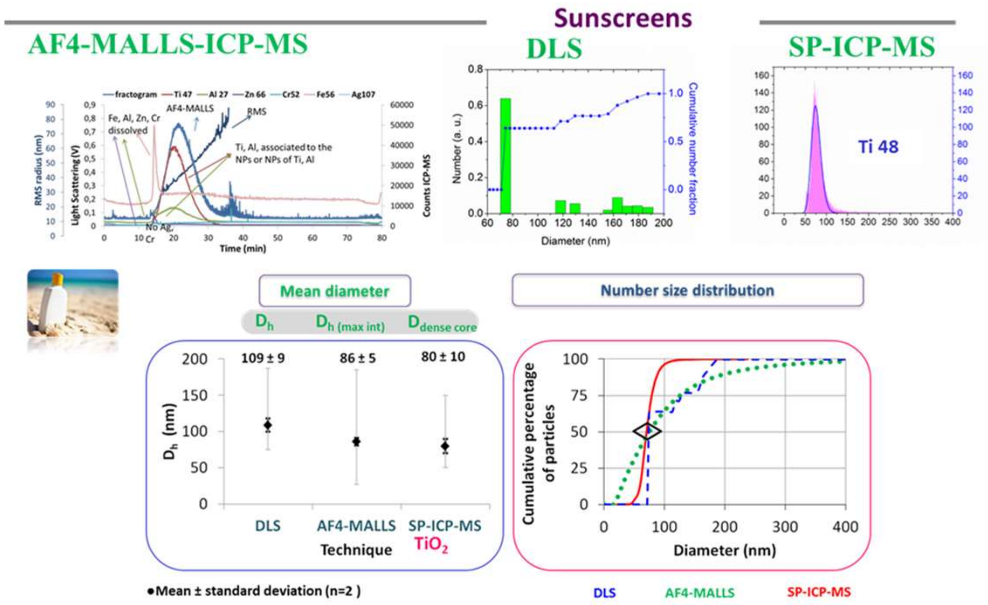
UNTWIN

Cross technique comparison for method validation

Intercomparison using the techniques available at the UT2A laboratory

Characterization of TiO2 particles in sunscreens

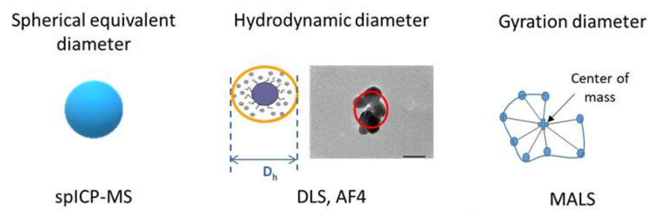


Characterization of particles in foods & cosmetics

Sample	Diameter(nm)	Composition	Technique
Candies	124 ± 8 147 ± 3 125 ± 4	N/A TiO_2 TiO_2	DLS AF4-MALLS-ICP-MS spICP-MS
Chewing gum	112 ± 4 216 ± 6 126 ± 1	N/A TiO_2 TiO_2	DLS AF4-MALLS-ICP-MS spICP-MS
Decorative pearls	35 - 225 30 - 150 50- 200	N/A Ag Ag	DLS AF4-MALLS-ICP-MS spICP-MS
Sunscreens	109 ± 9 86 ± 5 80 ± 10	N/A TiO_2, Al_2O_3 TiO_2	DLS AF4-MALLS-ICP-MS spICP-MS
Toothpaste	70-125 106 ± 9 50 - 100	N/A SiO_2, TiO_2 TiO_2	DLS AF4-MALLS-ICP-MS spICP-MS
Lip balm	45-175 50 - 90 40 - 100	N/A TiO_2, Al_2O_3 TiO_2	DLS AF4-MALLS-ICP-MS spICP-MS

Results agreed for most samples

Some differences related to the measurand associated with the analysis technique



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Chewing gum E171 – TiO_2 NPs

Particle size number distribution between with spICP-MS and SEM

Ultra Traces Analyses Aquitaine

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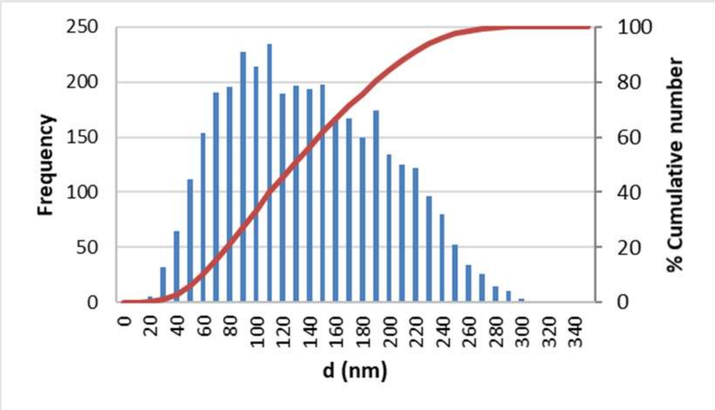
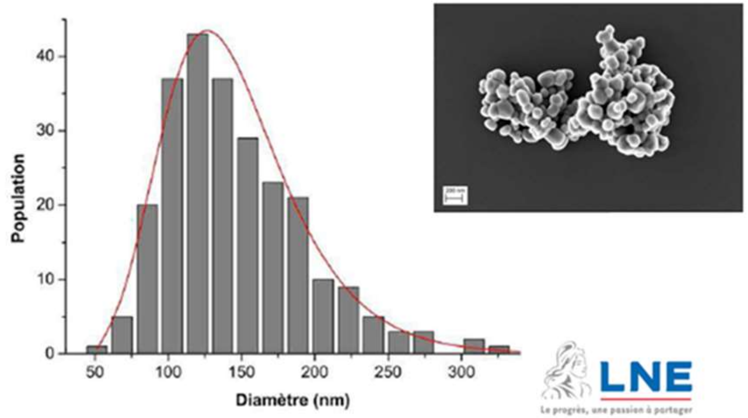
sp-ICP-MS (screening)



1700 – 3500 analysed particles / run

(n = 6)	Dmean (nm)	Dmedian (nm)	% < 100 nm	D10 (nm)
Mean ± SD	140 ± 3	135 ± 4	29 ± 2 %	65 ± 2

MEB (confirmation)



250 analysed particles

Dmean (nm)	Dmedian (nm)	% < 100 nm
137	127	23 %

Good agreement between the results obtained by these two techniques

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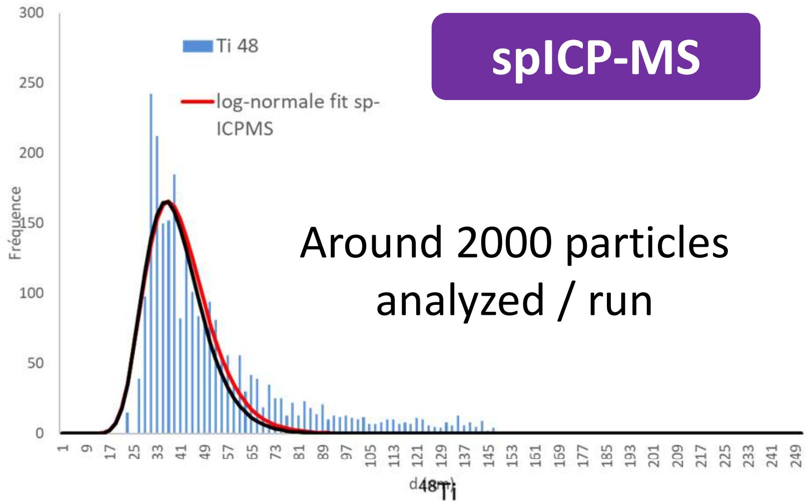
Golden Pepper E171- TiO2 NPs

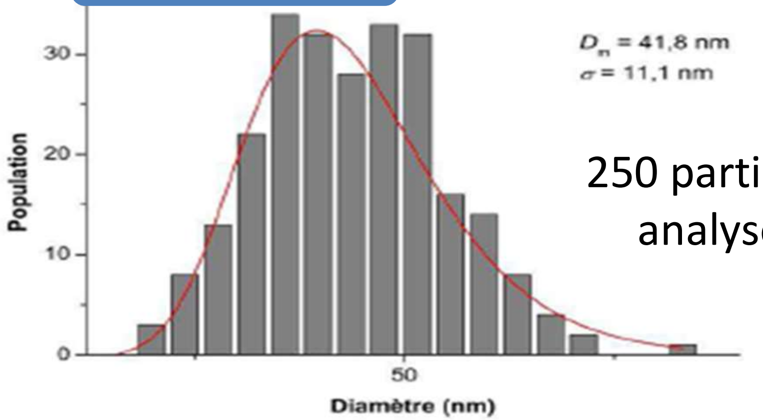
Particle size number distribution between with spICP-MS and SEM

spICP-MS

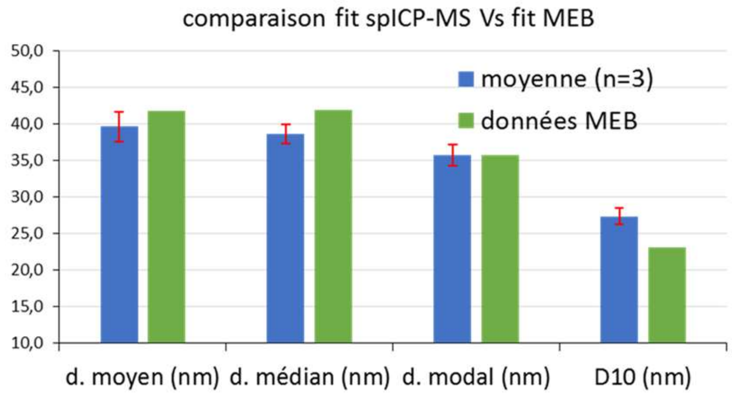


Around 2000 particles analyzed / run

SEM



250 particules analysed



Good agreement between the results obtained by these two techniques

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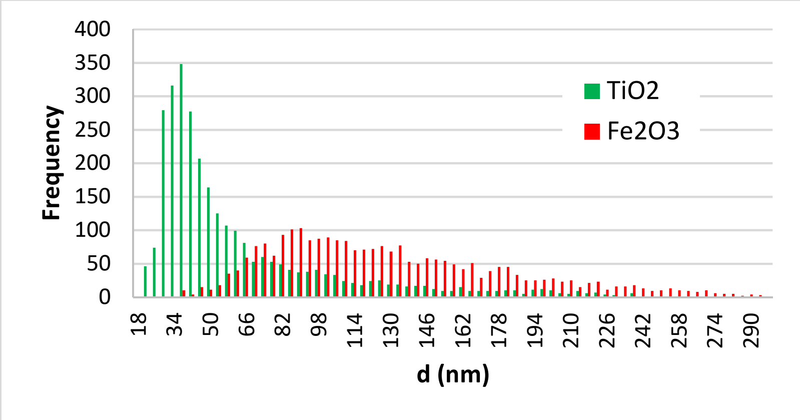
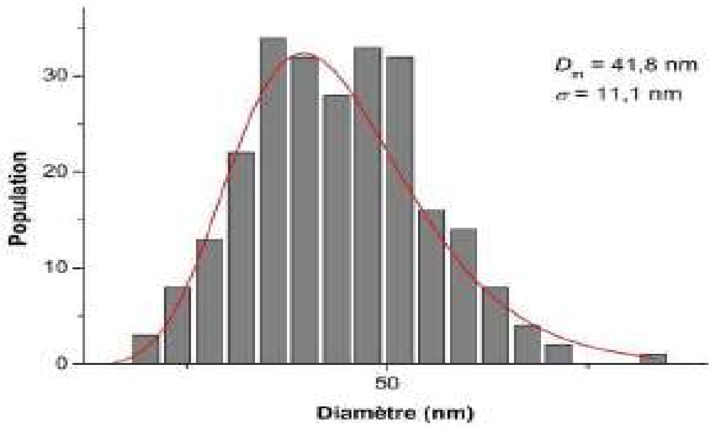
Golden Pepper E171 & E172 - TiO_2 & Fe_2O_3 NPs

Particle size number distribution between with spICP-MS and SEM





Parameter	Technique		
	spICPMS (Ti)	spICPMS (Fe)	SEM
Mean Diameter(nm)	**69**	**134**	**42**
Median Diameter (nm)	**46**	**121**	**42**
% of particles < 100 nm	**83**	**34**	**100**

No distinction between TiO_2 and Fe_2O_3 by SEM without an elemental analyzer (EDX)

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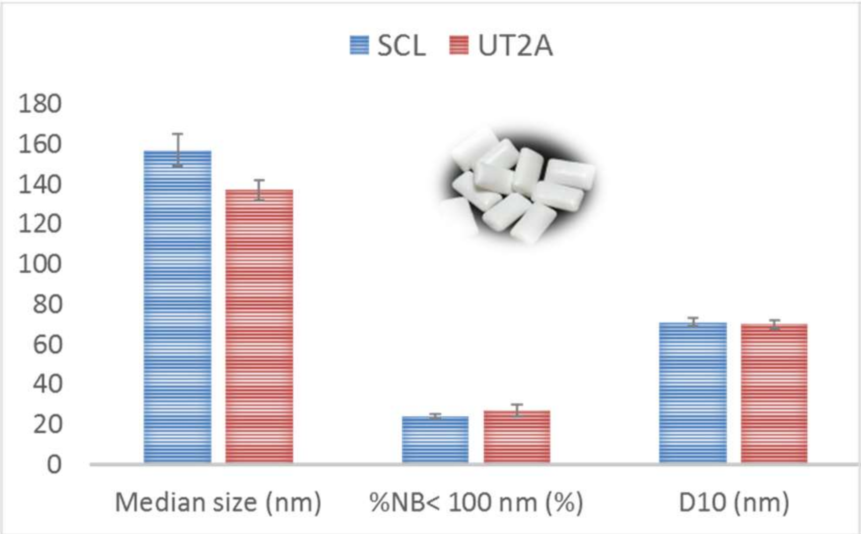
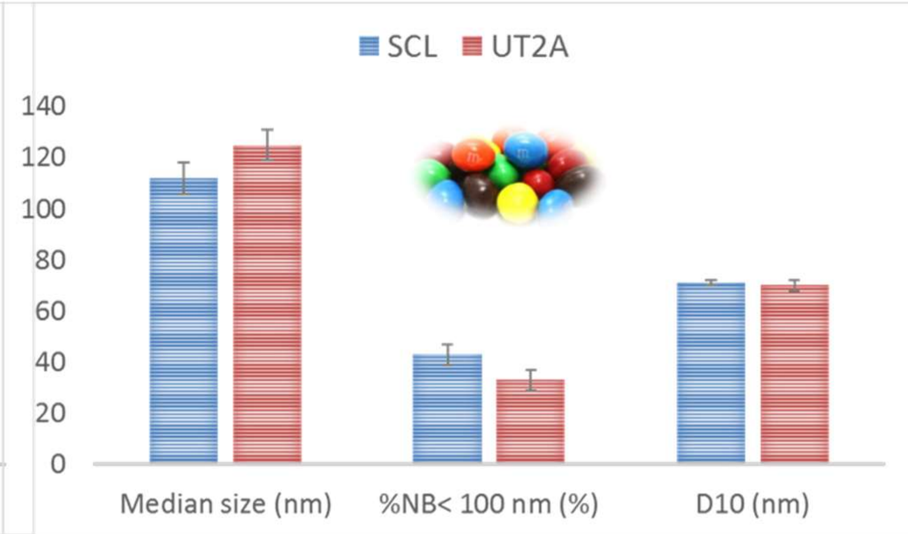
Inter-laboratory comparison

Sample preparation:
Sample + Ultrapure Water
Sonication (bath ; 10 min)





Good agreement between the different laboratories

To be completed with other laboratories and other samples and matrices

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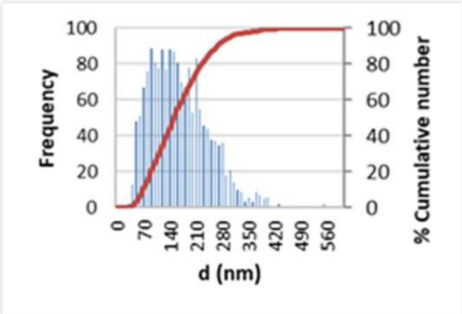
UNTWIN

Comparison between pristine additive & Food product

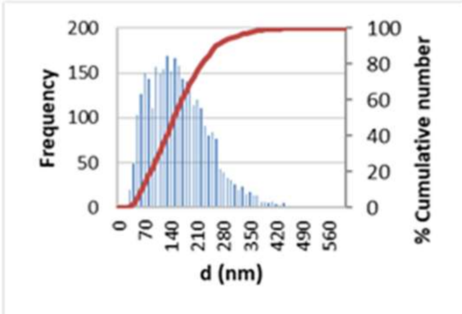
Liste d'ingrédients : Sorbitol E-420 Titanium Dioxide E-171 Xanthan E-415

Sirop "Bubble gum"



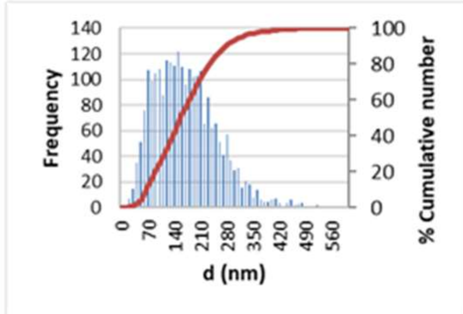
Mean (nm)	Median (nm)	% < 100 nm	D10 (nm)
166	154	23%	70

Sirop "noix de coco"



Mean (nm)	Median (nm)	% < 100 nm	D10 (nm)
163	152	24%	72

Additif "E171"



Mean (nm)	Median (nm)	% < 100 nm	D10 (nm)
171	160	22%	70

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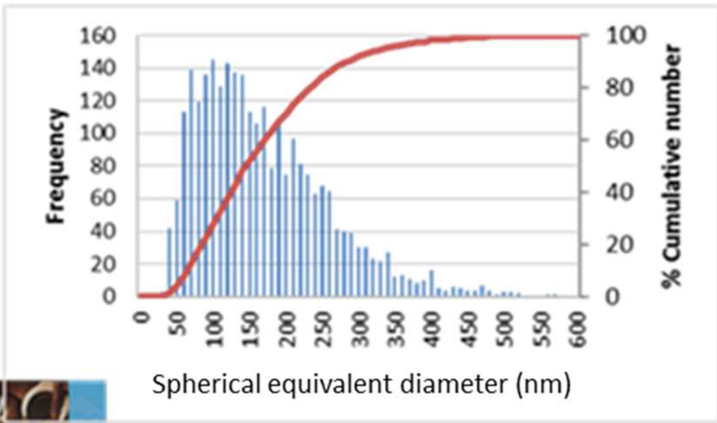
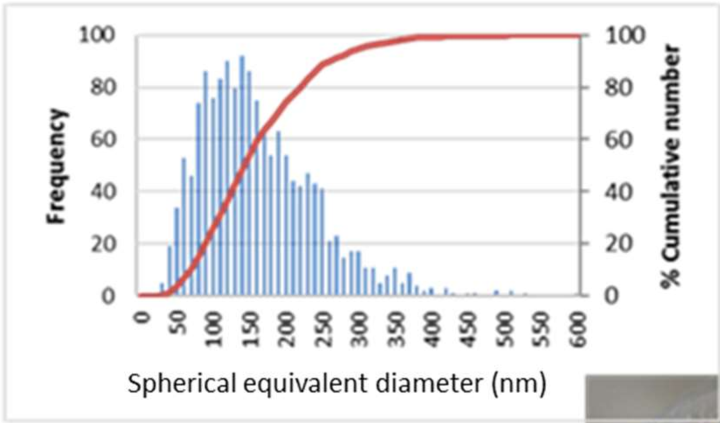
Comparsion between pristine additive & Food product

Almond dragees



E171 used for almond dragees



Parameter	Almond Dragee	E171
Mean Diameter (nm)	164 ± 3	164 ± 5
Median Diameter	147 ± 2	150 ± 4
% < 100 nm	27	23

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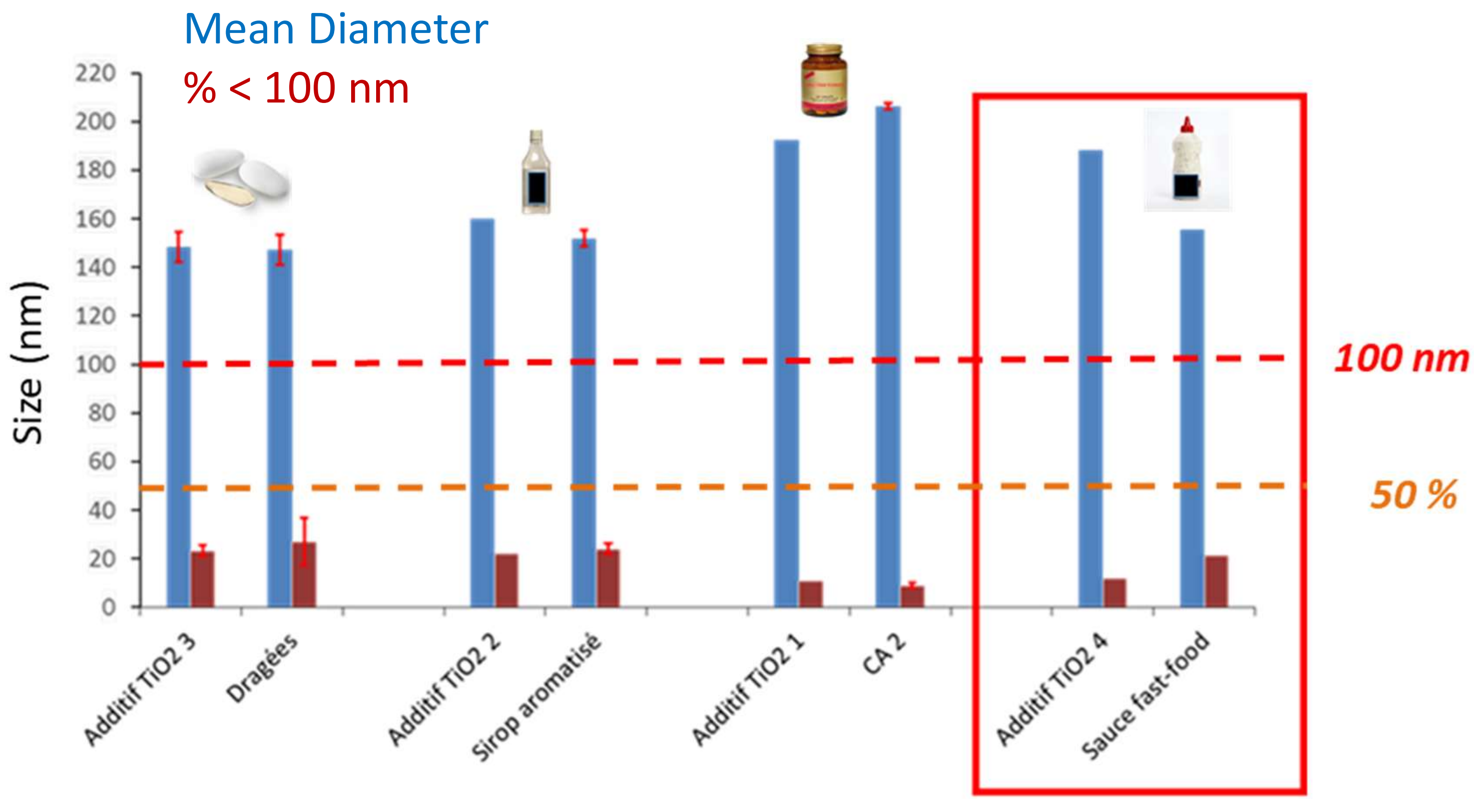
UNTWIN

Comparsion between pristine additive & Food product



Agreement between size distributions of the ingredient and the finished product

In some cases differences are observed : Sample preparation optimization required

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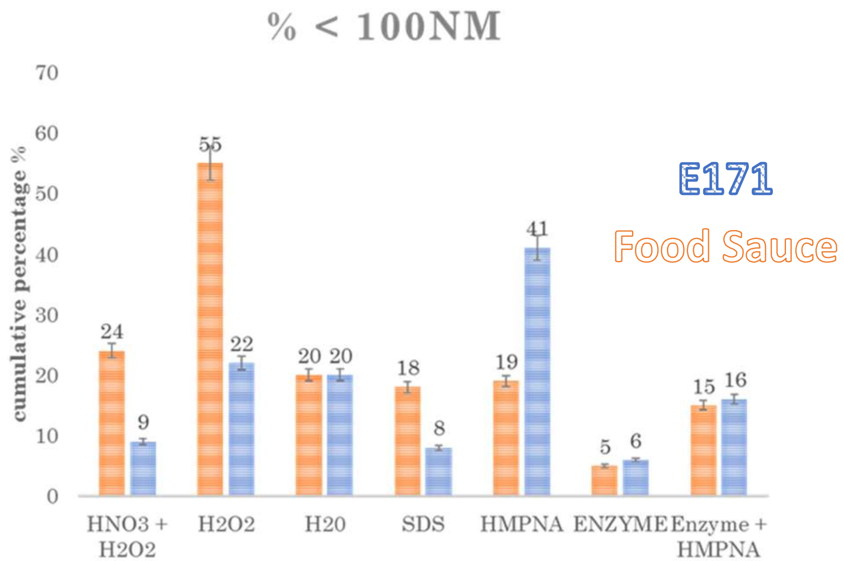
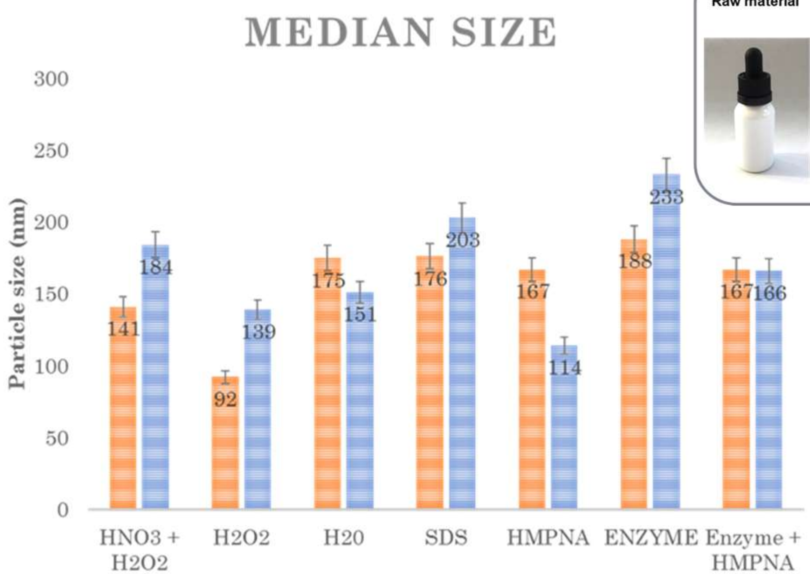
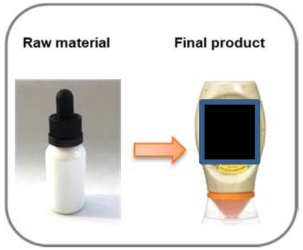
Optimization of TiO_2 NPs extraction in complex food samples

Influence of the extraction media on the granulometric properties of particles







Wide disparity in particle size results according to the extraction/dispersion media

What representativeness? Sample preparation must:

- preserve/not modify/denature the initial particle size
- be quantitatively representative

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UNTWIN

Sample preparation

As always, sample preparation is the key step, especially for "nanos"!

Main steps or processes used

- dispersion/extraction: ultrapure water, organic solvents, surfactants, alkaline solutions, etc.
- agitation: vortex, bath or ultrasonic probe (define time, duration, program, etc.)
- filtration, centrifugation

No miracle and universal recipe
Lack of a harmonized extraction method
Matrix/sample specific

Need to evaluate the respective influences of the different steps on particle size and recoveries

Implementation of a global methodology for method validation

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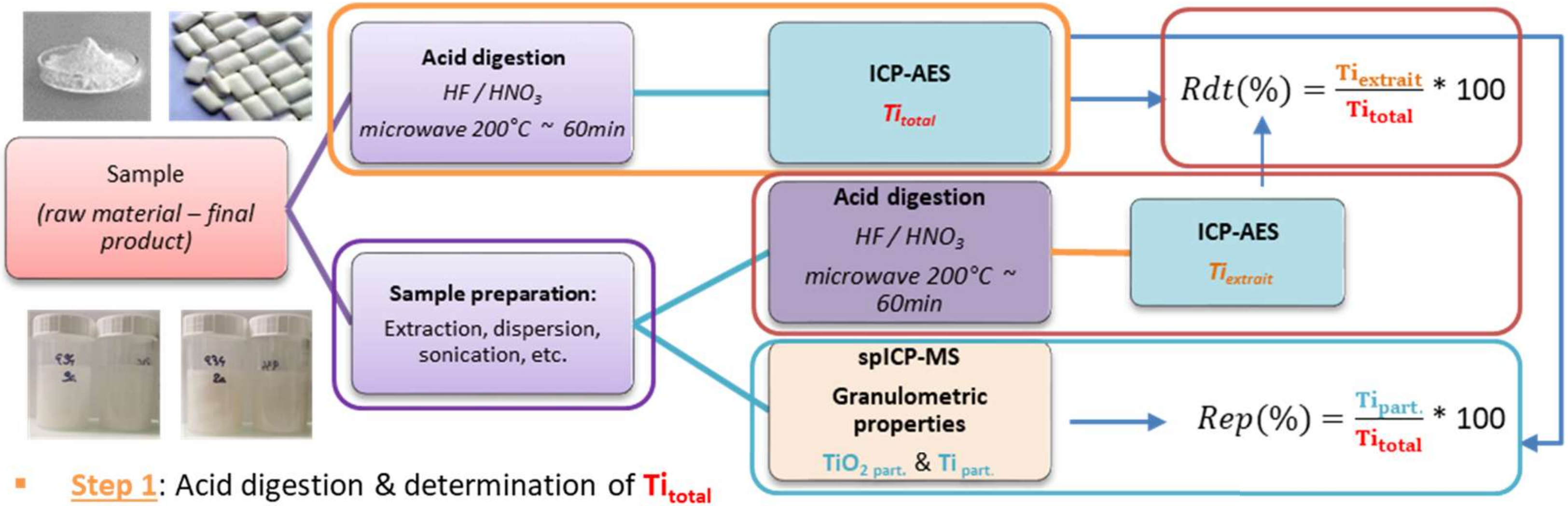
Sample preparation



- Step 1: Acid digestion & determination of Ti_{total}
- Step 2: Sample preparation for spICP-MS analysis
- Step 3: Acid digestion of the extract prepared for spICP-MS, determination of $Ti_{extrait}$ & determination of the exctraction revovey $(Rdt(\%))$
- Step 4: Analysis by spICP-MS of the extract, determination of TiO2 particles size distribution and concentration $TiO_{2\ part.}$ and $Ti_{part.}$ & evaluation of the extract representativity $(Rep(\%))$

Repeat steps 3 and 4 before and after each preparation process implemented to assess and quantify their respective influences
Necessary approach in the context of a method validation

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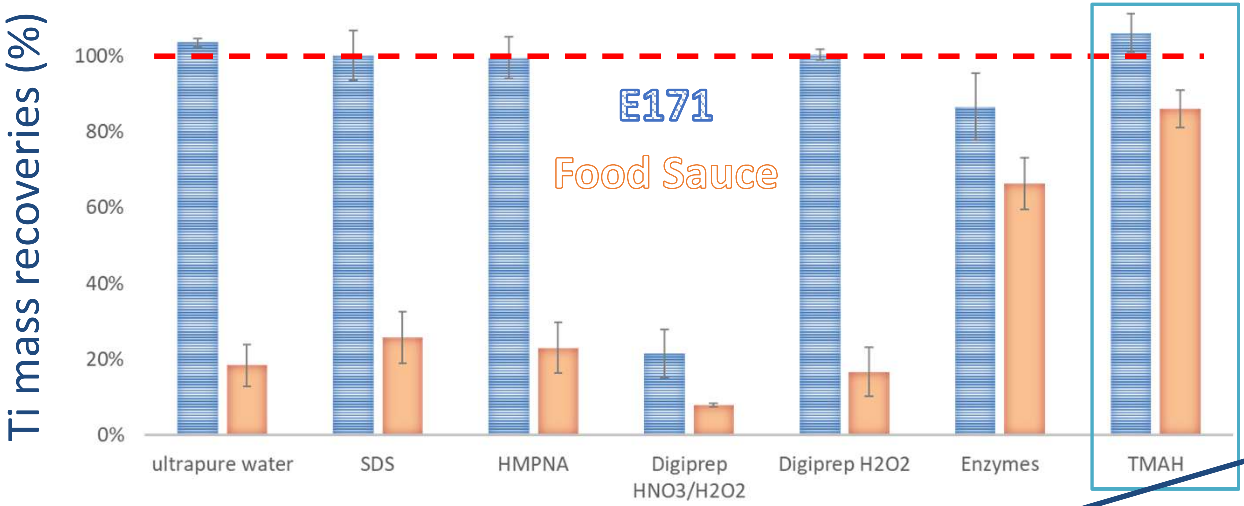
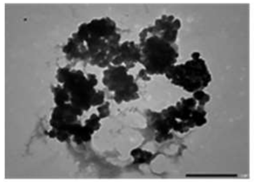
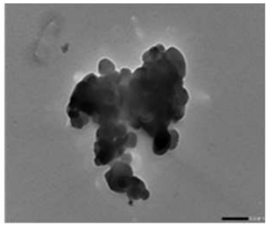
Optimization of TiO2 NPs extraction in complex food samples

Determination of Ti mass recoveries



Results consistent with TEM





Extraction	Sample	Median size (nm)	Mean Size (nm)
TMAH (pH 13)	TiO_2 ingredient	188 ± 2	205 ± 1
	Sauce kebab	176 ± 6	192 ± 7
ENZ (pH 7)	TiO_2 ingredient	273 ± 23	289 ± 23
	Sauce kebab	278 ± 9	297 ± 9

ENZYMATIC :
Extraction yield of the order of 70% for the sauce
High NPs size: probable formation aggregates/agglomerates

TMAH (2.5%):
Extraction yield of the order of 80% for the sauce
Limits the risk of aggregate/agglomerates

Complementary parameters to optimize: TMAH concentration and volume, sonication step (bath or probe, duration, program), etc.

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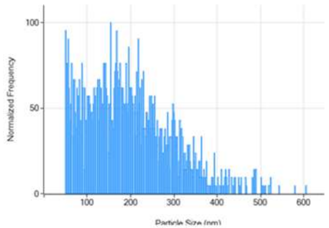
UNTWIN

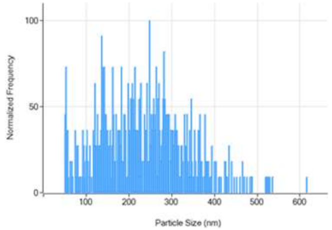
Evaluation of the method accuracy

Spiking experiment (n = 3)

- E171 (ingredient sauce)



- NM 100



Sample	Median size (nm)
NM-100 RM	220 ± 24
E171	197 ± 5

\+ 

sample	Median size (nm)	Recovery (%)
Spiked mayonnaise NM-100 RM	180 ± 2	99 ± 9
Spiked mayonnaise E171	173 ± 5	93 ± 5

- ✓ Enhancement of particle dispersion in the presence of the matrix
- ✓ Good recoveries

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Method implementation on food sausages

SAMPLE	Country	E171 (label)	Fat content (g / kg)	Total Ti (g/kg)	TiO_2 particles concentration (g/kg)	NPs fraction (% < 100nm)	Median size (nm)	Ti recovery (%)
White sauce	France		260	< 0.001	NA	NA	NA	NA
			260	< 0.001	NA	NA	NA	NA
			290	< 0.001	NA	NA	NA	NA
	Spain	x	< 5	2.8 ± 0.1	4.0 ± 0.3	7.5 ± 0.1	211 ± 2	86 ± 3
Mayonnaise	France		720	< 0.001	NA	NA	NA	NA
			710	< 0.001	NA	NA	NA	NA
	Spain	x	0	0.69 ± 0.04	1.15 ± 0.08	6 ± 1	255 ± 1	100 ± 1
		x	< 5	5.5 ± 0.2	6 ± 1	2.3 ± 0.3	321 ± 7	65 ± 6
	UK	x	0	0.86 ± 0.04	1.4 ± 0.2	6 ± 1	247 ± 7	99 ± 7
Aïoli	Spain	x	0	1.18 ± 0.04	1.1 ± 0.1	3.6 ± 0.3	280 ± 1	56 ± 3
Cocktail sauce	Spain	x	< 1	0.55 ± 0.02	0.77 ± 0.02	12 ± 1	213 ± 10	84 ± 1
Caesar sauce	UK	x	0	0.9 ± 0.1	0.92 ± 0.02	8.6 ± 0.4	206 ± 9	61 ± 1
White chocolate	UK	x	0	0.60 ± 0.02	0.6 ± 0.2	7 ± 1	231 ± 19	60 ± 10

- No TiO_2 particles detected in French fatty food samples, as indicated in the label
- Significant amount of TiO_2 (0.6 – 6 g/kg) in dietetic samples from UK and Spain

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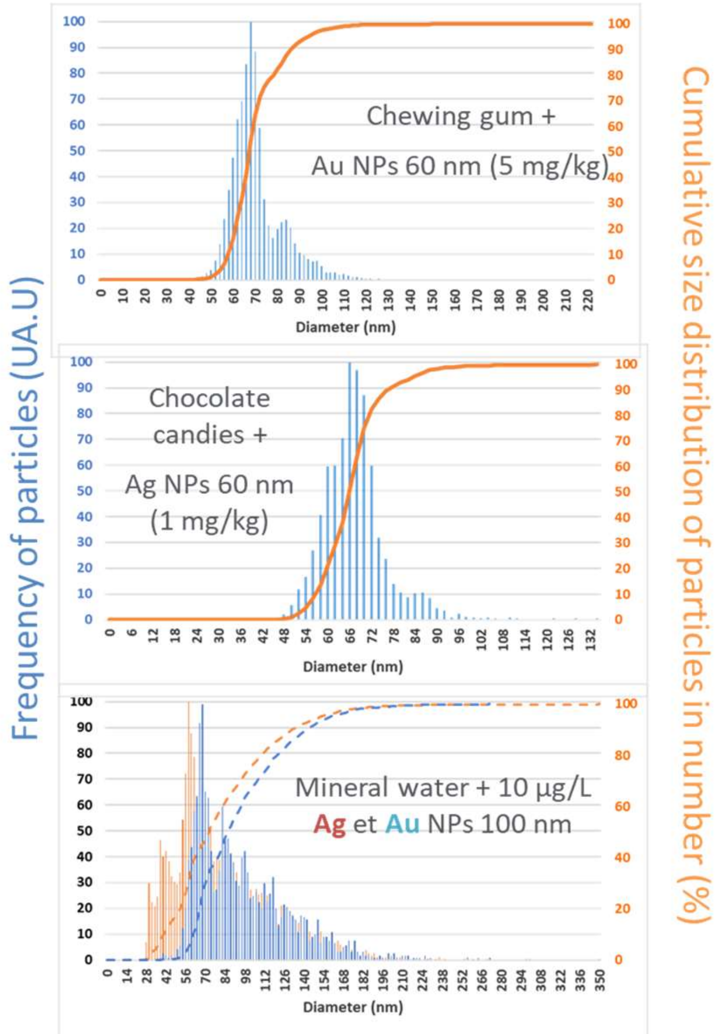
Other examples of sample spiking for method validation



Parameter	Results
Mean Diameter (nm)	65 ± 2
Recovery (%)	95 ± 2

Parameter	Results
Mean Diameter (nm)	62 ± 3
Recovery (%)	91 ± 3

Parameter	Results
Mean Diameter Ag NPs (nm)	86 ± 8
Mean Diameter Au NPs (nm)	93 ± 1
Recovery Ag NPs (%)	101 ± 4
Recovery Au NPs (%)	94 ± 3

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spICP-MS : Suited tool for characterization of most of inorganic particles

Complementary to confirmation techniques (TEM, SEM, SEM-EDX, etc.)

Relatively new and booming technology

Many developments still to be made to meet all needs

Technical and instrumental limitations (spherical model, 1 element / analysis, LOQ of some elements, etc,)

Many developments to be made on the development of sample preparation protocols for complex matrices

Questions?

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Thank you for your attention!

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