Assessing the potential risks of silver nanoparticles in antimicrobial applications, using miniaturized flow field-flow fractionation and multi-angle light scattering

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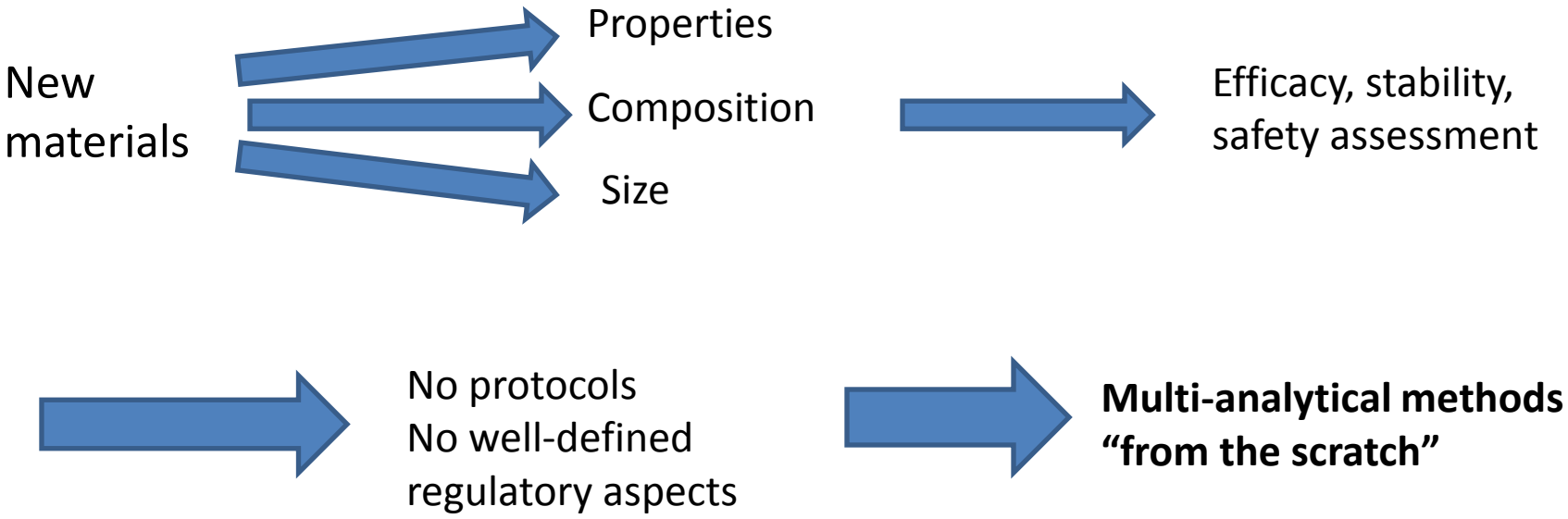
New materials, new protocols



Together with new synthesis and production techniques, analytical platforms have to be developed accordingly

Metal releasing particles: a challenging characterization

"Only very little is known about the rate of dissolution of silver nanoparticles.

> As this rate directly determines **the concentration of silver ions in** the vicinity of a nanoparticle, it is highly important for any antimicrobial application of silver nanoparticles, and also for **assessment of the toxicity** of silver nanoparticles in humans. In addition, the final fate of silver nanoparticles that are released into the environment depends on these data.

It is likely that the rate of dissolution depends not only on the chemical species but also on **the particle size**, the surface functionalization, and the particle crystallinity.

In addition, the temperature and the **nature of the immersion medium** (e.g., the presence of salts or biomolecules) will be major factors"

"…The **toxicity of nanoparticles** in the body and in the environment is currently under intense discussion and investigation..."

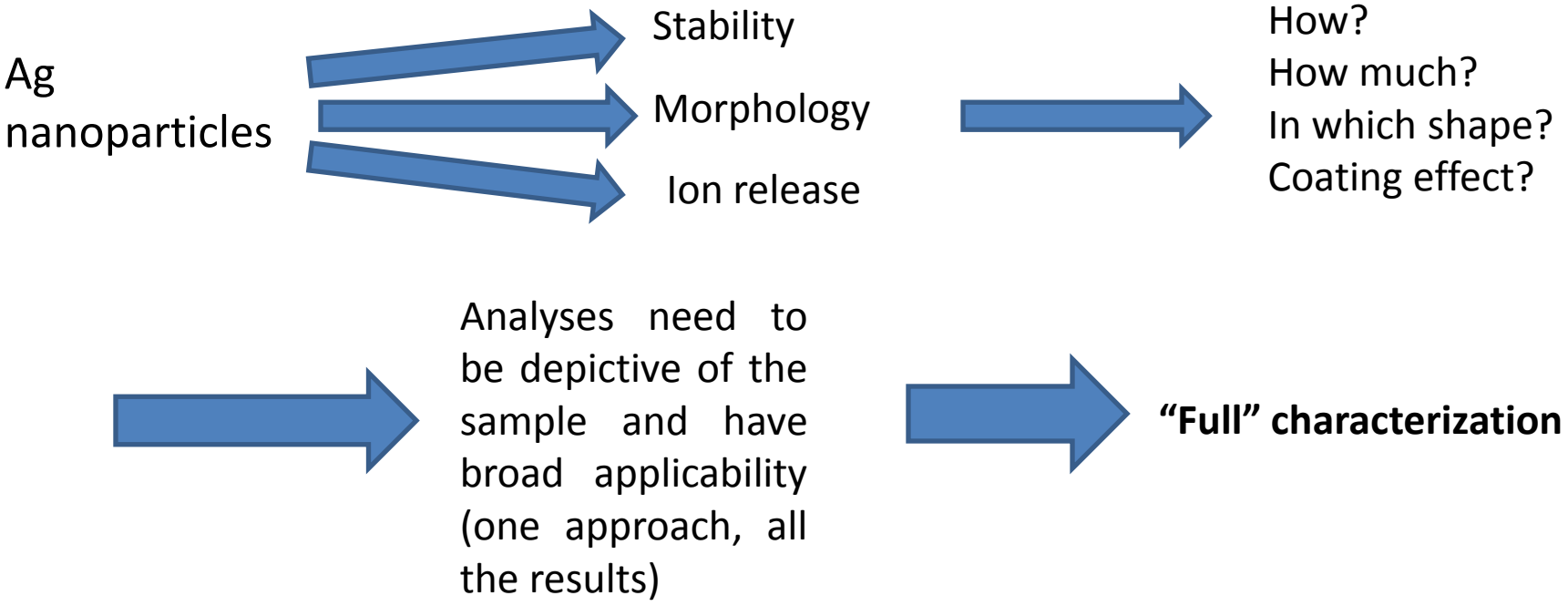
"…The biological action of **freshly prepared and aged nanoparticles** is strongly different due to the different amounts of released ions..."

"..Unfortunately, the dissolution in a biological medium is much more complicated to measure and describe because of the presence of various compounds in the medium, and the fate of the released silver ions is also unclear."

"……published **discrepancies** in reported toxicological level……."

S. Kittler et al., Chem. Mater. 2010, 22, 4548–4554
DOI:10.1021/cm100023p

Silver nanoparticles and their features



AgPVP nanoparticles (nanosol), pristine and SiO_2 coated

Silver Nanosol

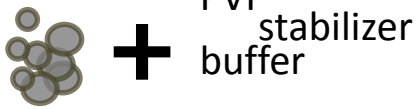
Good antibacterial activity against many type of bacterial strains (e.g. Escherichia coli, Staphylococcus Aureus) → production of antimicrobials based on AgPVP nanoparticles

Citotoxicity comparable to antimicrobial activity → Necessity of detailed study of ionic silver's fate from production to waste

Aqueous colloidal nanosuspension (nanosol) of Ag-PVP nanoparticles was provided by Colorobbia SpA (Italy).

content

Product composition and formulation

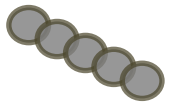


Activity

behaviour

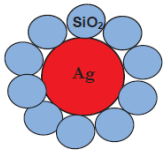
Shape and aggregation state

Behavior in different media



Changes over time

Coating with other compounds (SiO2)



Complexity of sample → Necessity of product separation -besides characterization in different condition- to assess efficacy and address safety issues

4548 *Chem. Mater.* **2010,** *22,* 4548–4554
DOI:10.1021/cm100023p

CHEMISTRY OF MATERIALS
Article

Toxicity of Silver Nanoparticles Increases during Storage Because of Slow Dissolution under Release of Silver Ions

S. Kittler,[†] C. Greulich,[‡] J. Diendorf,[†] M. Köller,*[,‡] and M. Epple*[,†]

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"The rate and degree of the dissolution of silver nanoparticles depend on their surface functionalization, their concentration, and the temperature. In a given system under given conditions,(….) the nanoparticles do not fully dissolve. ***This will change in a dynamic environment*** *(..).*

Such changes in the nanoparticle dispersions may escape the attention of the experimentalist because the classical analytical methods (e.g., dynamic light scattering, electron microscopy, or ultracentrifugation) ***are insensitive*** *to released ions and because* ***the particle diameter undergoes only a minor change****.*

A dynamic light scattering experiment of aged particles would typically be accepted as quality control that the particles did not change during storage, but this experiment would not reveal such dissolution phenomena."

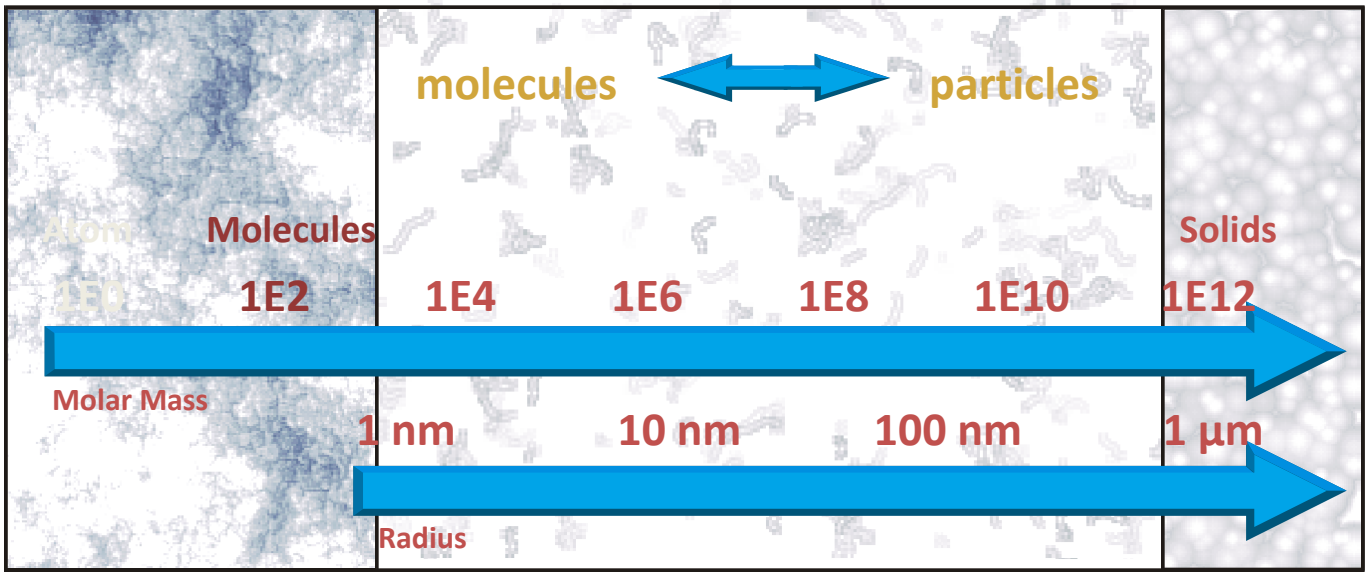
"Although the importance of silver ion in the biological response to nanosilver is widely recognized, ***the drug delivery paradigm has not been well developed for this system****, and there is significant potential to improve nanosilver technologies through* ***controlled release formulations****"*

Controlled Release of Biologically Active Silver from Nanosilver Surfaces

Jingyu Liu,[†] David A. Sonshine,[‡] Saira Shervani,[‡] and Robert H. Hurt[‡,§,*]

[†]Department of Chemistry, [‡]School of Engineering, and [§]Institute for Molecular and Nanoscale Innovation, Brown University, Providence, Rhode Island 02912, United States

Flow field flow fractionation: a soft and flexible separation technique



-**Macromolecules:** proteins, protein complexes, nucleic acids.
-**Nanoparticles:** polymers, metal nanoparticles, viruses, virus-like particles liposomes, lipoproteins, protein aggregates, subcellular components.
-**Microparticles:** large protein aggregates, whole cells.

Instrumental features

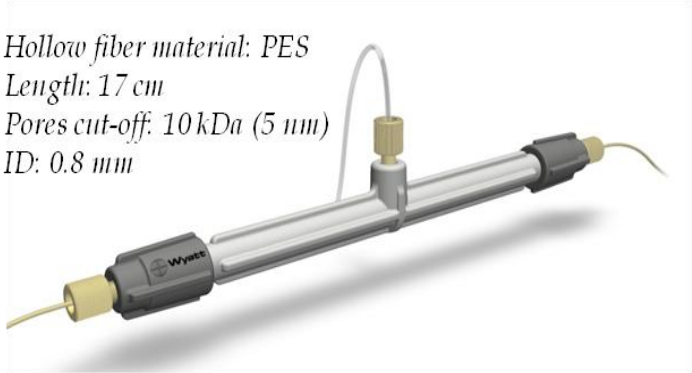
Hplc setup (degasser, pump, autosampler, UV-Vis detector)

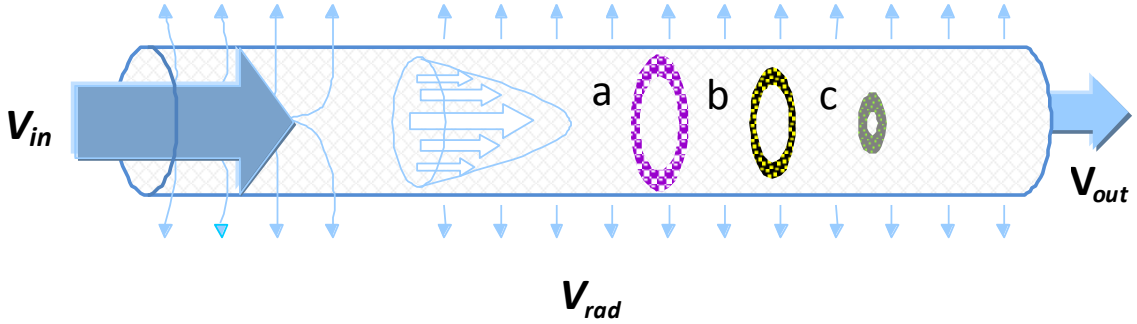
Flow Field flow fractionation

Multi Angle Light Scattering (MALS) detector

Hollow-fiber flow field flow fractionation (HF5)

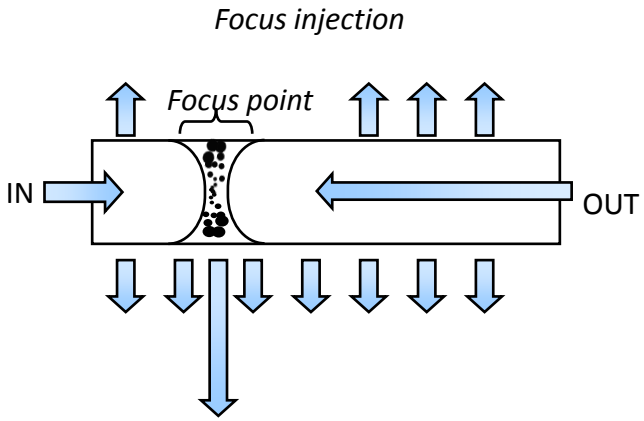
- Hollow fiber with defined porosity → 10kDa cutoff
- No stationary phase (gentle/native separation)
- Dimension-based separation in two steps
-Miniaturized (little sample dilution)





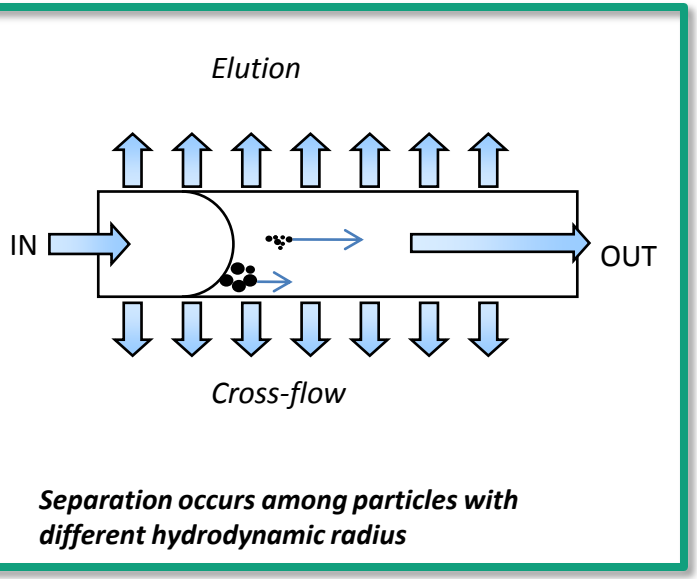
$$t_r = \frac{R_f^{\,2}}{8D} \ln\left(\frac{F_{in}}{F_{out}}\right)$$

Physical steps of a separation



The injected sample is focused in a narrow band prior to the analysis to improve separation

Smaller particles are filtered out of the fiber during this step

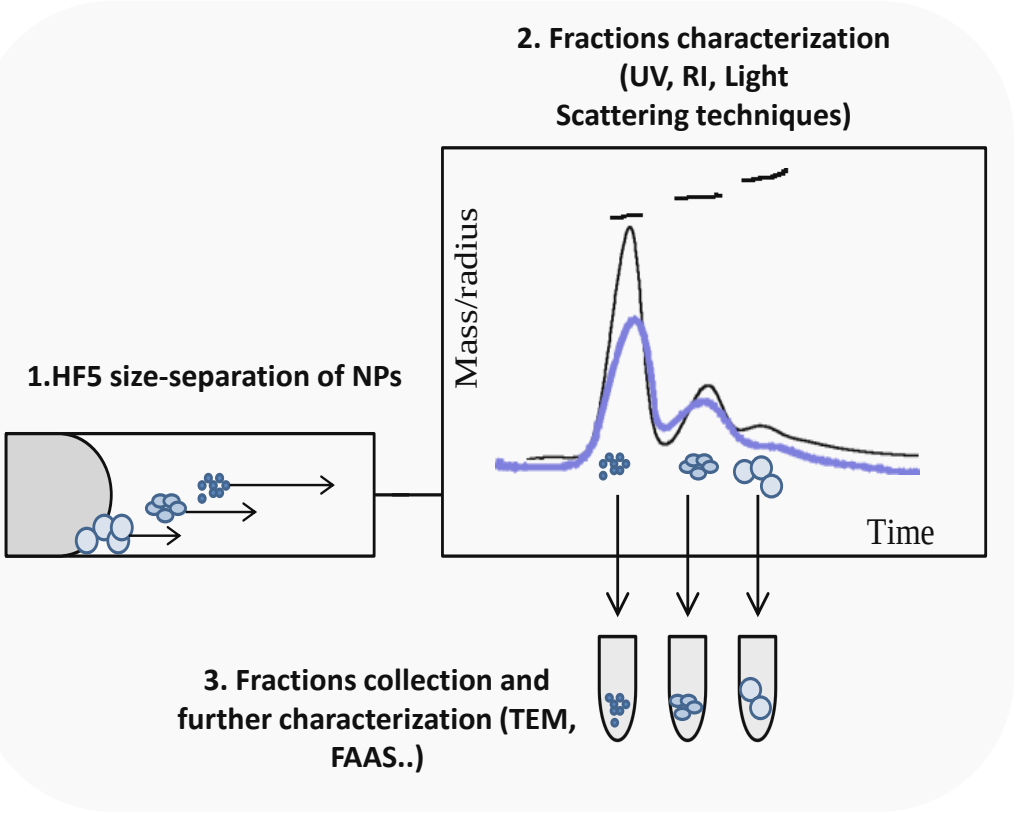


The applied hydrodynamic field (cross flow) allows for particles to be separated

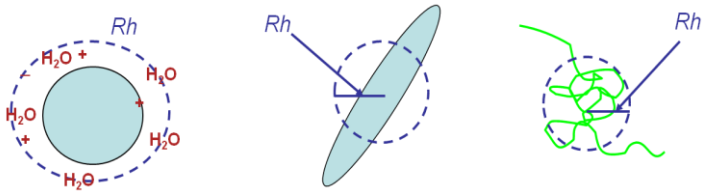
The different populations reach the detectors

Possibility of “non-conventional” use: filtration and filtered sample collection

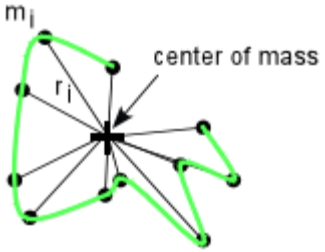
Analytical platform



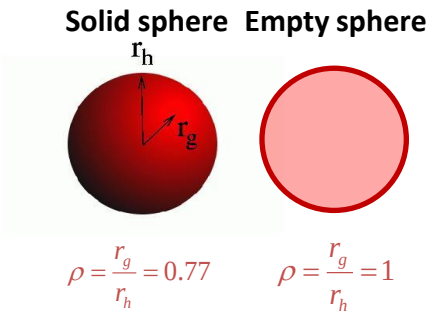
1. Separation based on hydrodynamic radius



2. Scattering intensity based on particles' compactness

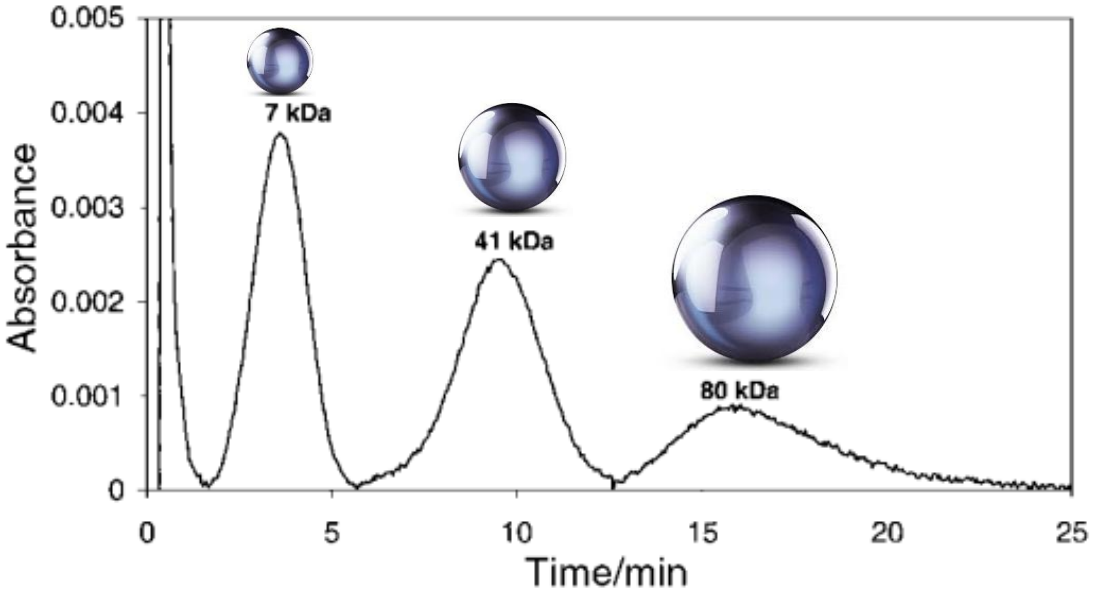


Results correlation:
Shape characterization



Information obtained

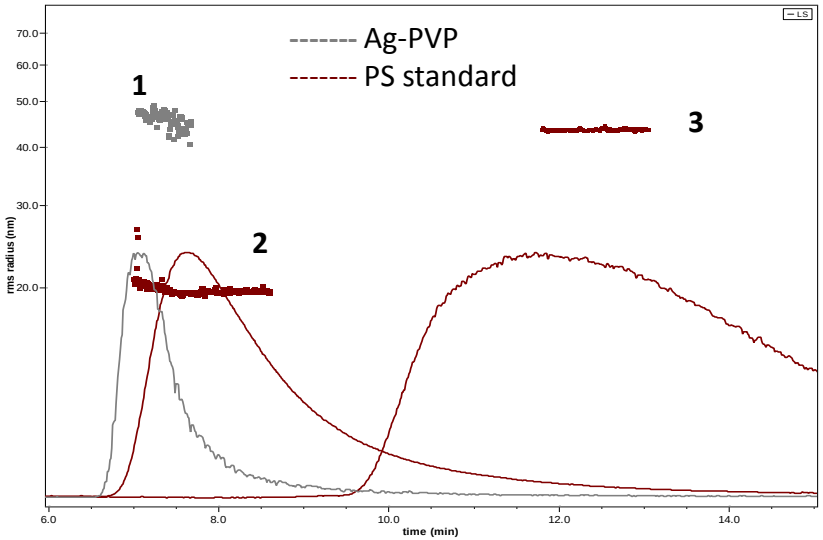
Retention time is linked to hydrodynamic radius → The slower, the bigger



Gyration radius is linked to compactness → The lower, the more compact

Rg1 > Rg2

Sample characterization and method calibration



Sample: silversol

Optimized method

Mobile phase: de-ionized water

Spherical standards injected under same conditions to calibrate the method

Same hydrodynamic radius of smaller standard
Same gyration radius of bigger standard → the particles are not spherical

→ **Rg/rh = 1.7 → rod conformation**

Gentle separation → preservation of **aggregates** (not clearly visible with other characterization techniques)

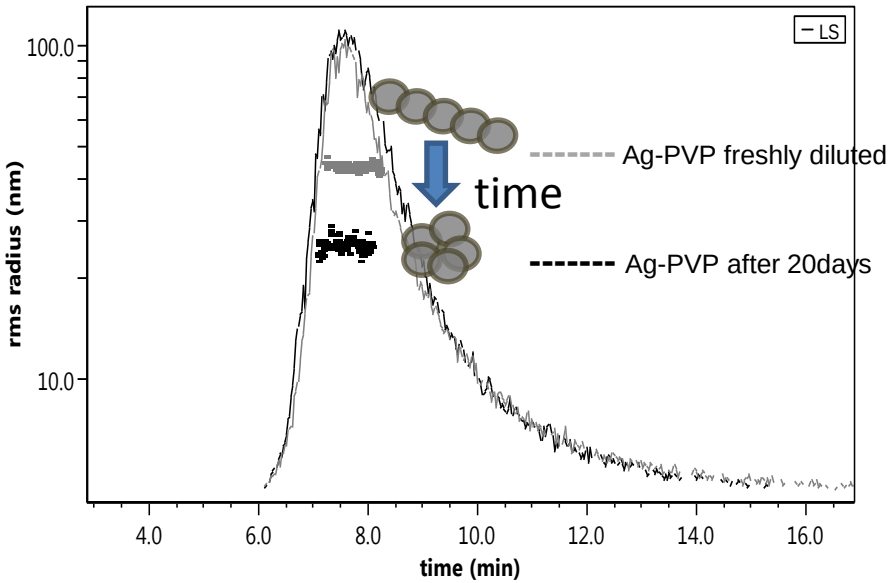
→ Nanoparticles in solution exist as chain-like aggregates

V. Marassi et al., Journal of Pharmaceutical and Biomedical Analysis 106 (2015) 92–99 DOI:10.1016/j.jpba.2014.11.031

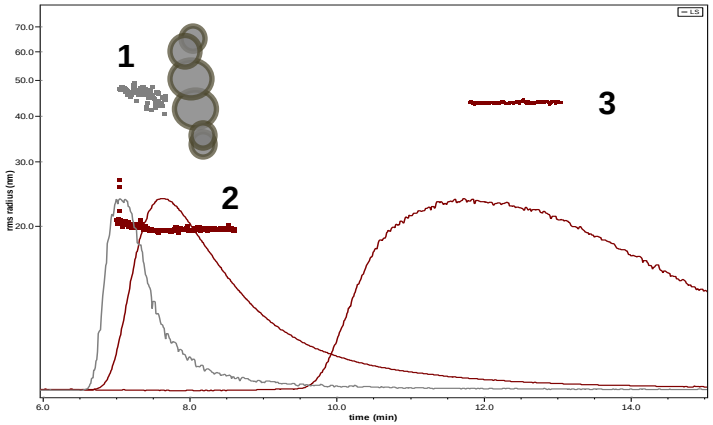
What we have studied so far

Particles have been
-diluted 10 times with H2O milliQ and analyzed after 20 days (right),
-analyzed using phosphate buffer as mobile phase (below)

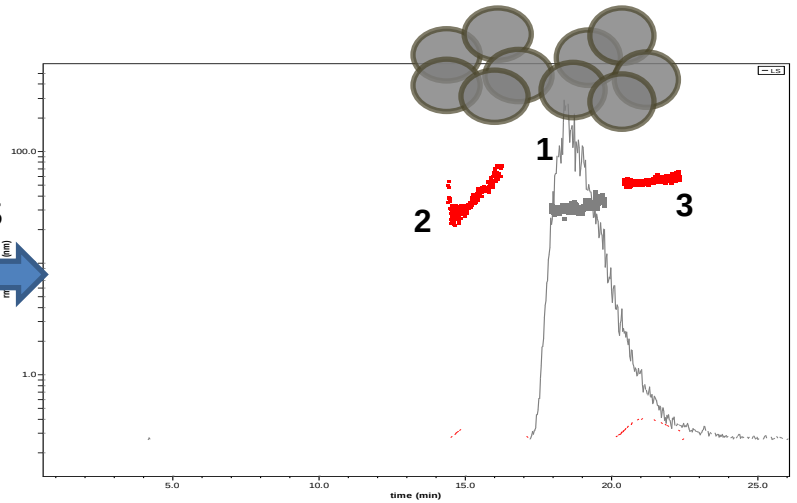
Stability over time…



…and in different media



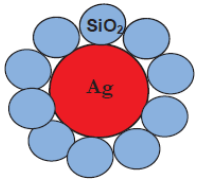
electrolytes



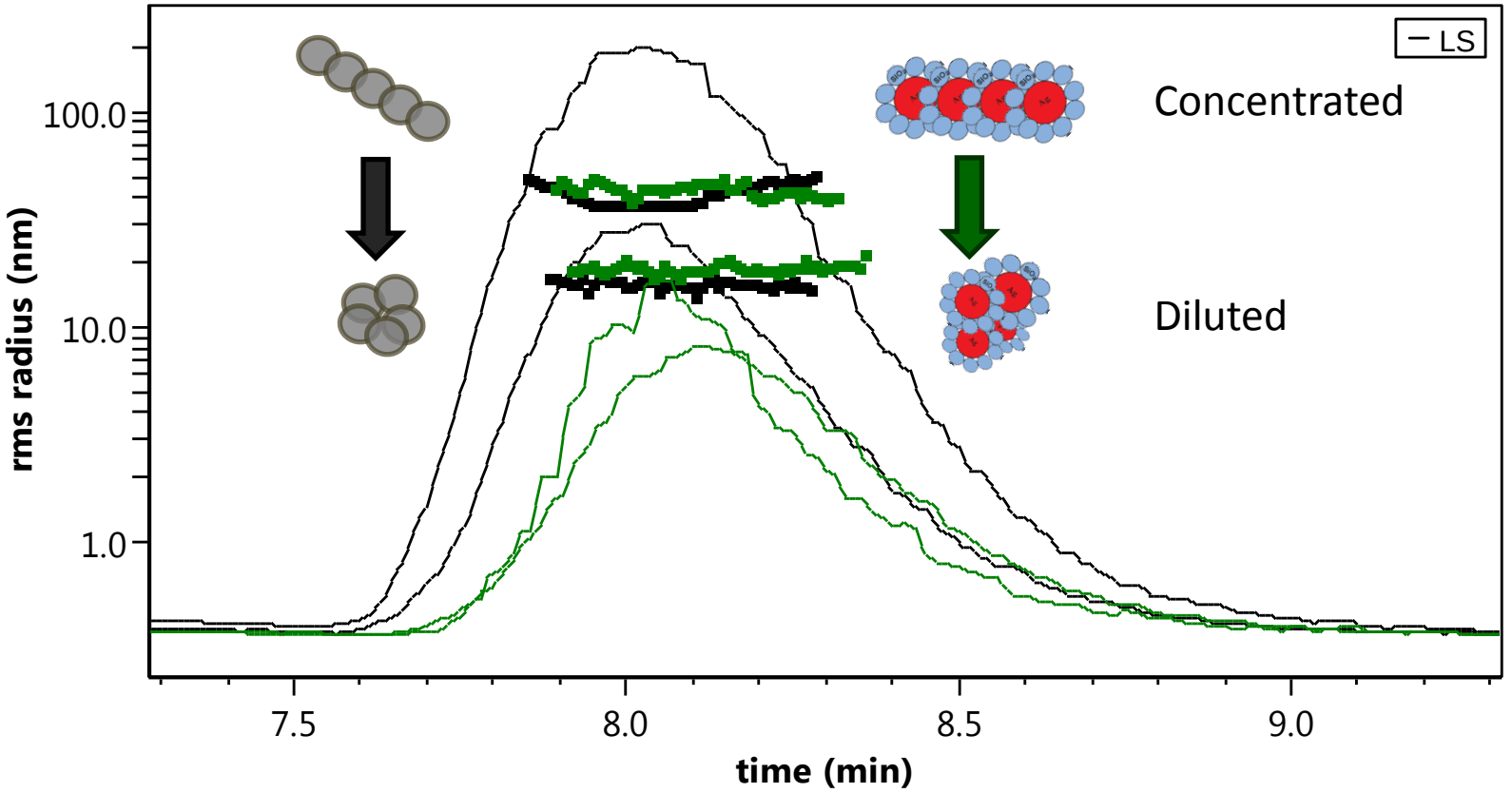
Coating effect on stability and metal release

-AgPVP *(synthesis: M. Blosi, S. Albonetti, M. Dondi, G. Baldi, A. Barzanti: "Process for preparing stable suspensions of metal nanoparticles and the stable colloidal suspensions obtained thereby" PCT/EP2010/052534 WO 2010/100107 A2, 2010)*

→ concentrated sample
→ diluted sample (10 times, with bidistilled water)

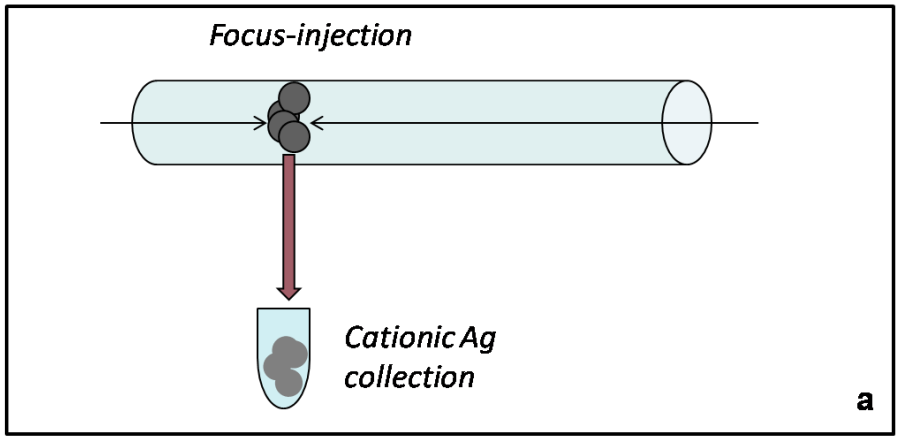


-SiO2 coated Ag PVP, Ag:SiO2 weight ratio 1:1, silica monolayer on Ag surface (heterocoagulation).
→ concentrated sample
→ diluted sample (10 times, with bidistilled water)

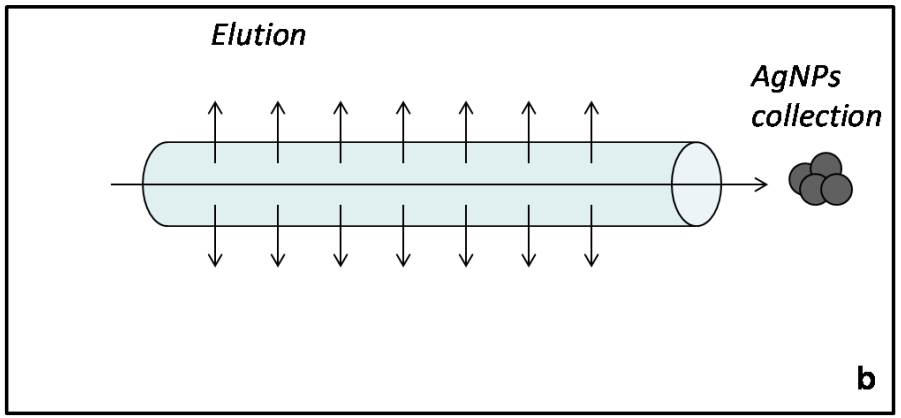


Effect of SiO2 coating vs effect of dilution

- Screening of AgNPs efficacy according to different goals (citotoxicity, antimicrobial activity)



Free Ag+ collection and quantification (AAS)



AgPVP particles isolation
-Test Ag+ release
-Obtain purified nanoparticles

Quantification of Ag+ fraction

One step analyses for Ag+ release study and nanoparticles isolation

Effect of SiO2 coating vs effect of dilution

Does SiO2 heterocoating regulate Ag+ release?

Does aggregation state influence Ag release?

ug tot	**Sample**	**Ug xflow**	% Ag^+
30	Ag0.03	11.97	**39.91068**
30	Ag0.3	16.5	**55.11122**
12	AgSi 0.012	4.1	**34.41439**
12	AgSi 0.12	5.7	**48.09488**

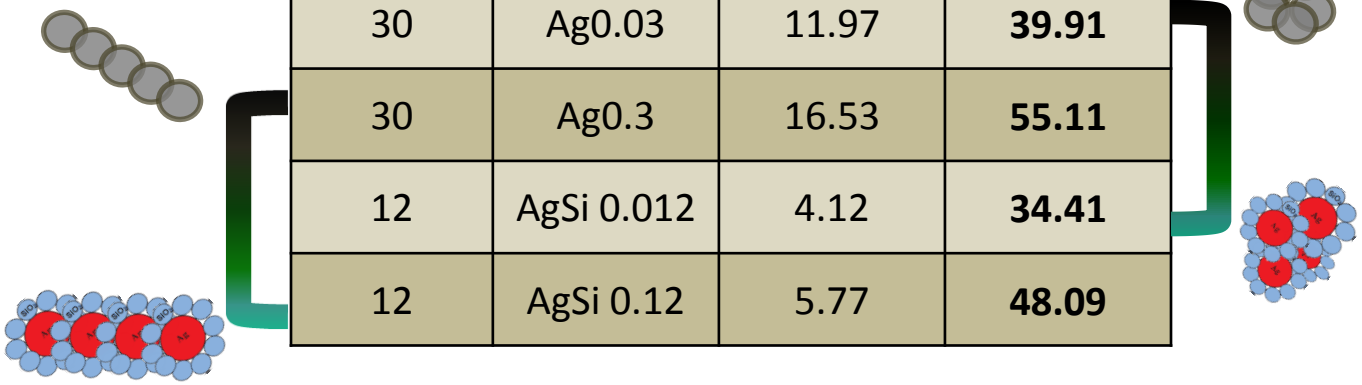
The more diluted samples have a lower percentage of free Ag!

→ Shape effect of particle aggregates

Effect of SiO2 coating vs effect of dilution

Does SiO2 heterocoating regulate Ag+ release?

Does aggregation state influence Ag release?



ug tot	**Sample**	**Ug xflow**	% Ag^+
30	Ag0.03	11.97	**39.91**
30	Ag0.3	16.53	**55.11**
12	AgSi 0.012	4.12	**34.41**
12	AgSi 0.12	5.77	**48.09**

=> A lower surface/volume ratio makes Ag less available to enter the solution

Dilution → shape changes → Ag release modulation

One technique, no sample preparation, all the answers

Conclusions

- characterization of silver nanoparticles in native conditions leads to understanding of their release mechanism
- coating and dilution effects can be studied with a simple and non destructive technique
- ionic silver collection and shape characterization can be achieved with a single analysis

Future work

- Systematic shape-Ag release correlation → towards a faster method

- Isolation of AgPVP particles →synthesis of particles with customized shape

-Purification of nanoparticles → destabilization and coating tests

- Screening of candidates for protein corona to exploit drug carrier potentiality

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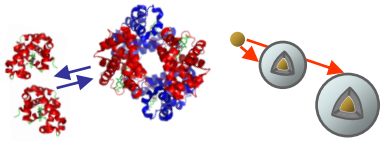
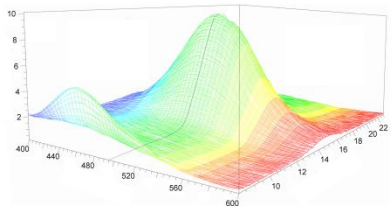
...everything flows by

byFlow we do...





Development of custom-fit methodologies for quality control (QC) of nano/biotech materials to be outsourced to QC labs which employ the same instrumentation.

Methods development and customized analyses for clients which manufacture nano/biotech materials.

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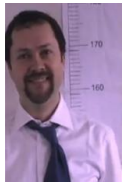

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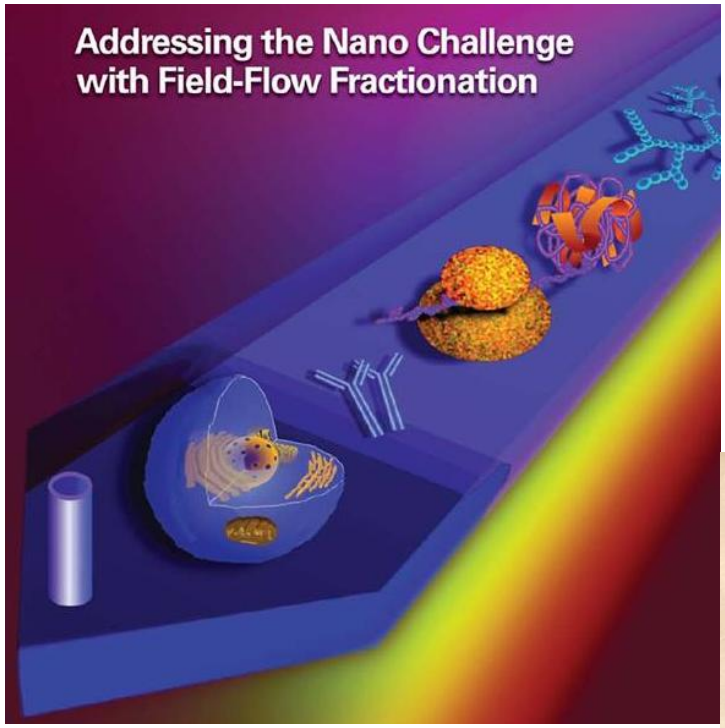

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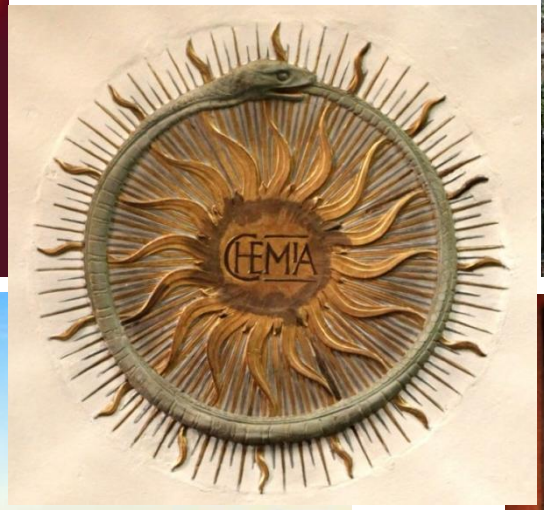
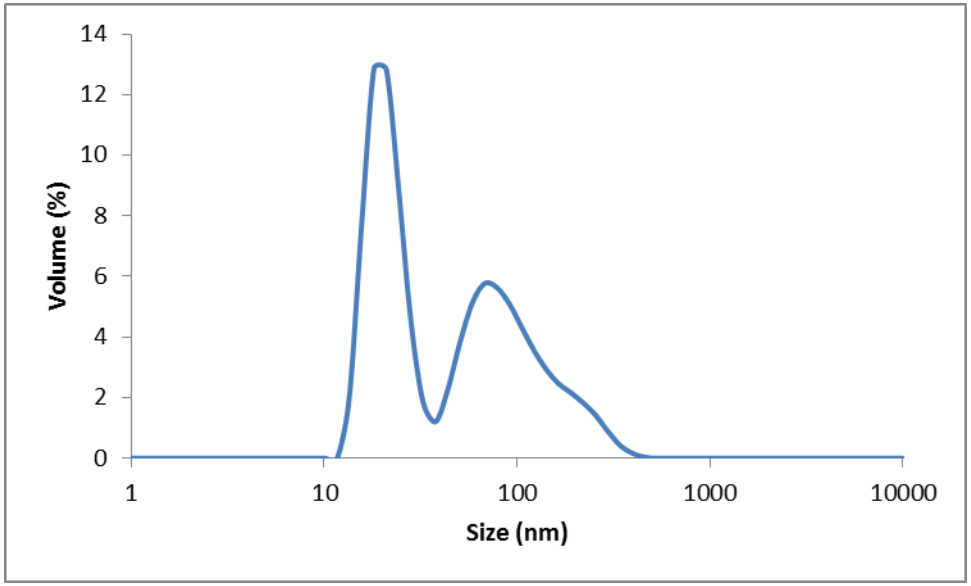
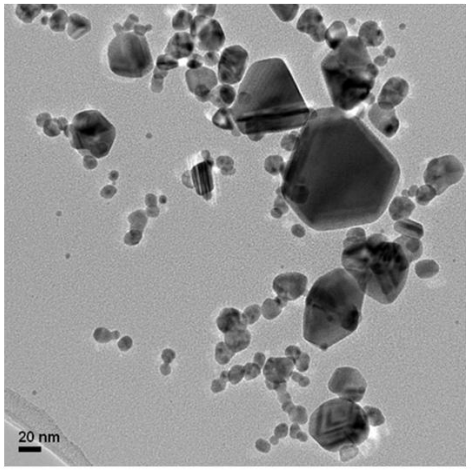


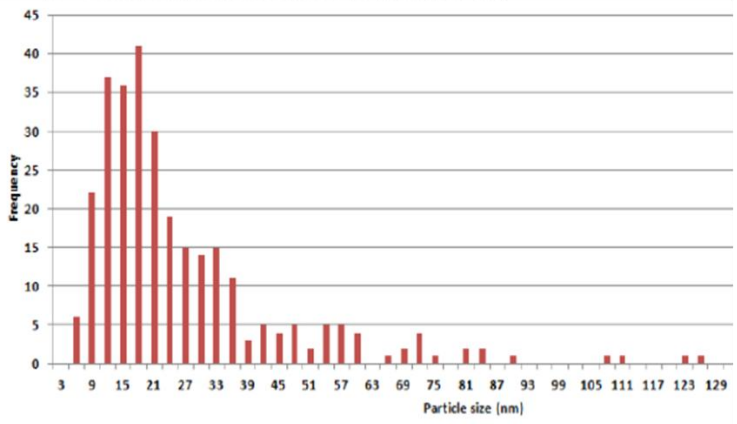
Comparison with other techniques



DLS



TEM

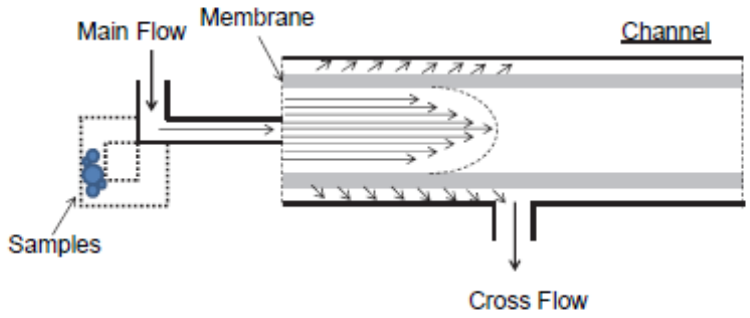


Cons

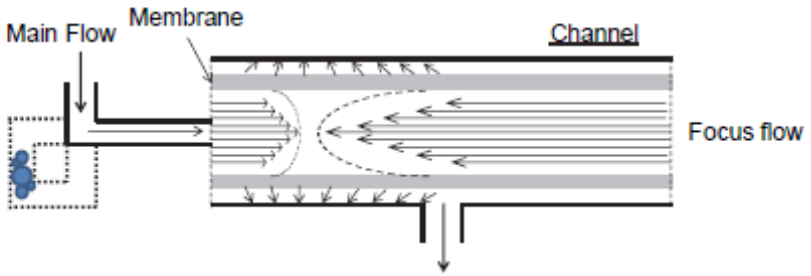
- No separation of the sample
- Sample treatment (deposition, drying) (TEM)
- Difficult estimation of populations' abundance (DLS)
- Necessity of combining DLS and TEM results to obtain the whole picture
- Loss of information about sample in solution
- Loss of information about sample in different media

Full scheme of an HF5 method

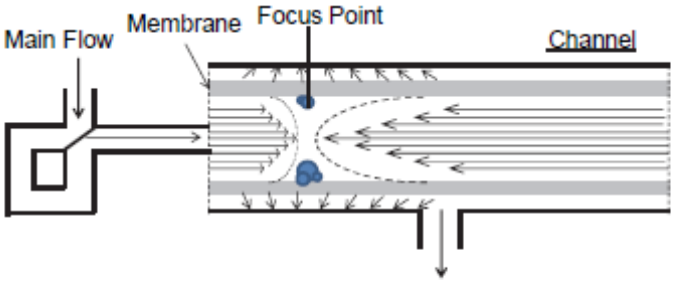
Elution



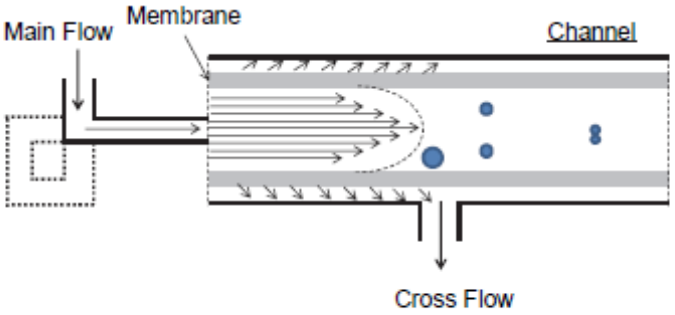
Focusing



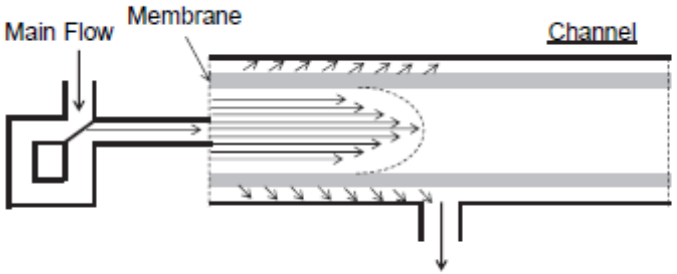
Focusing + Injection



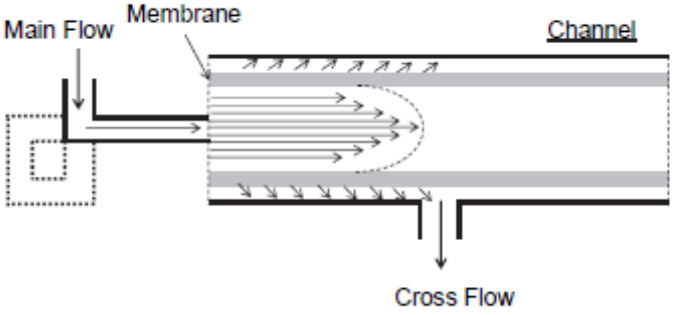
Focus flow



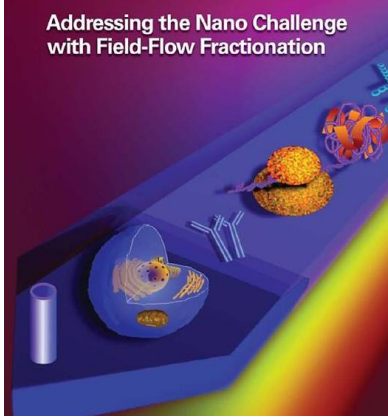
Elution + Injection (wash)



Elution



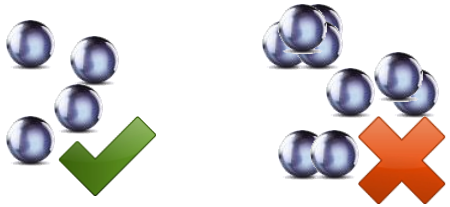
Valentina Marassi, Chemistry Department 'G. Ciamician' – University of Bologna



Separation science → Both for R&D and commercial nanoproducts

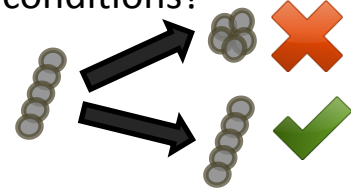
HF5-UV-MALS
"A *solution* for your *solution*"

Is it monodispersed?



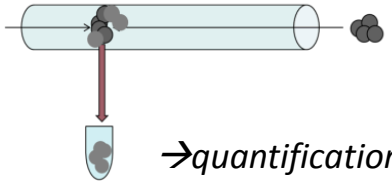
Aggregation study

Is it stable over time/in these conditions?



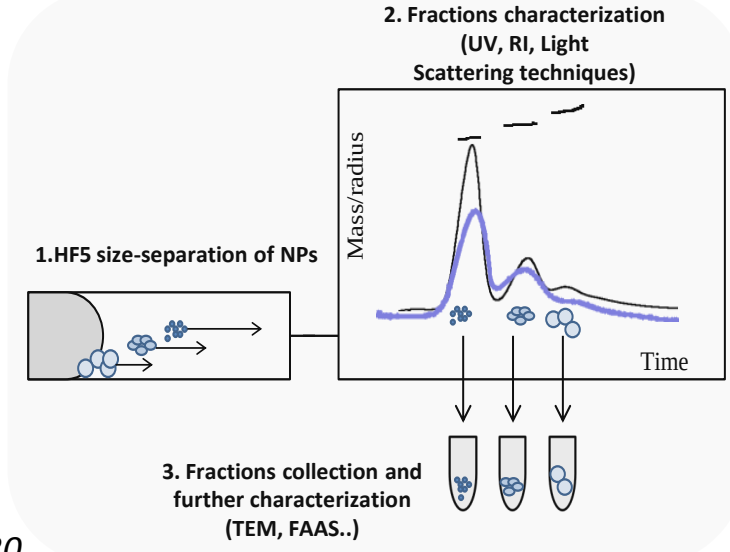
Stability/formulation study

Does it release its active compounds?



Release study

Non -destructive
All-in-one analyses
No sample handling/preparation
Time saving
Cost effective



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Learn more @ Parallel Session 3A, Tue 10th, 12.30