Particle size analysis by Light scattering
Dynamic (DLS)
& Static (SLS)

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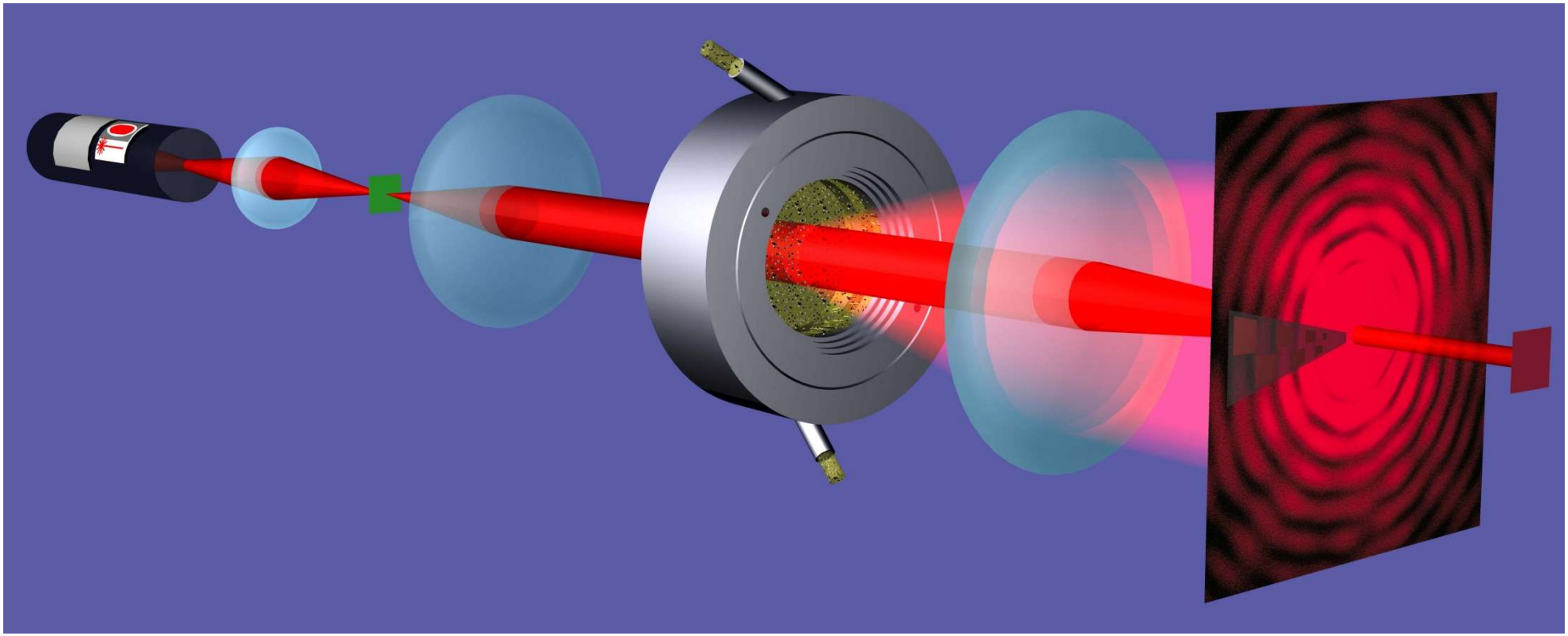
Particle size analysers by laser diffraction

MASTERSIZER **3000**

- Size range: 10 nm – 3,5 mm

Particle size analysis by laser diffraction

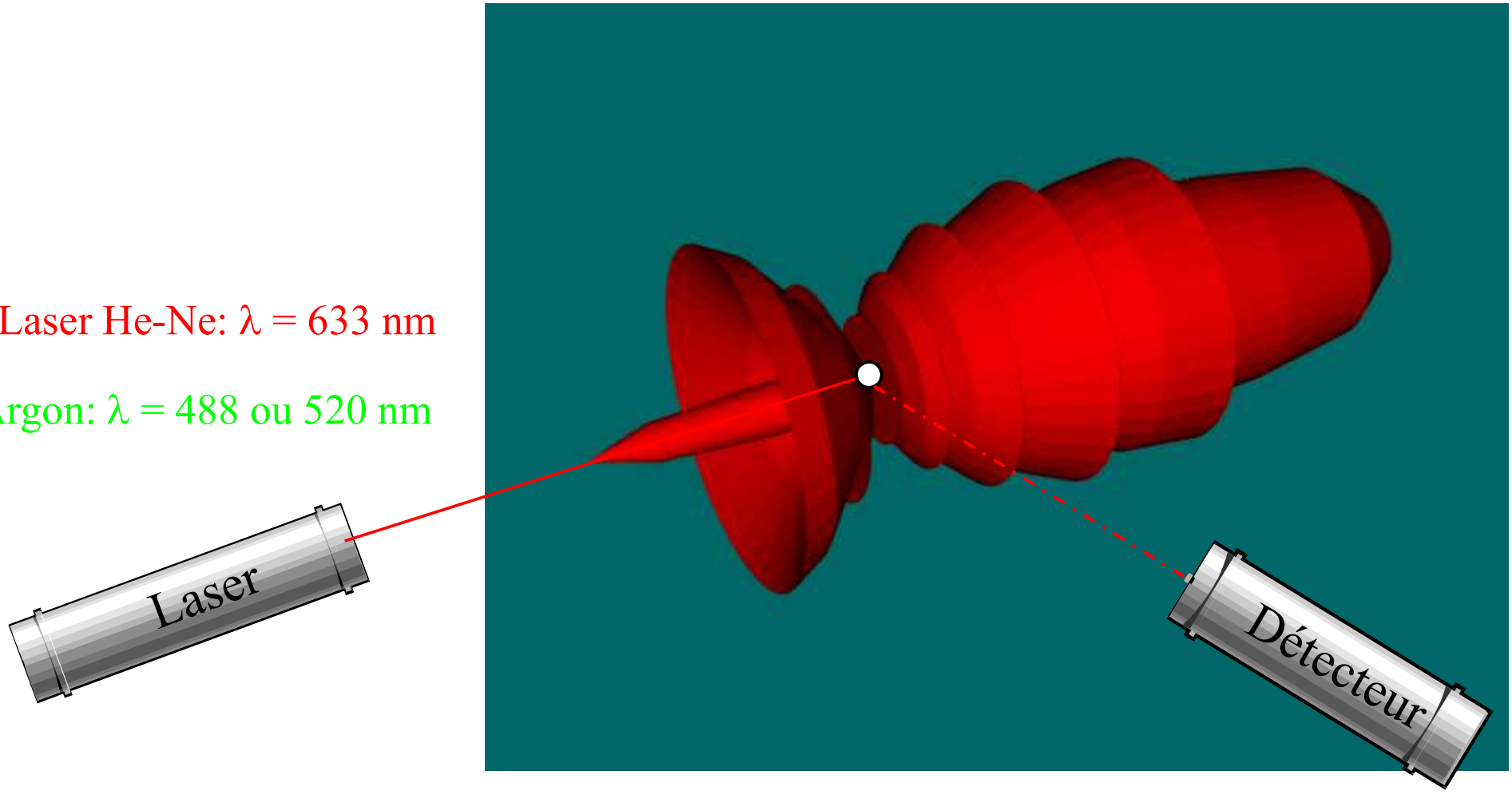


- Flow cell: 1 to 100ml in 100ml or 1 liter

Interactions between light and particles

Laser He-Ne: $\lambda = 633$ nm

Argon: $\lambda = 488$ ou 520 nm



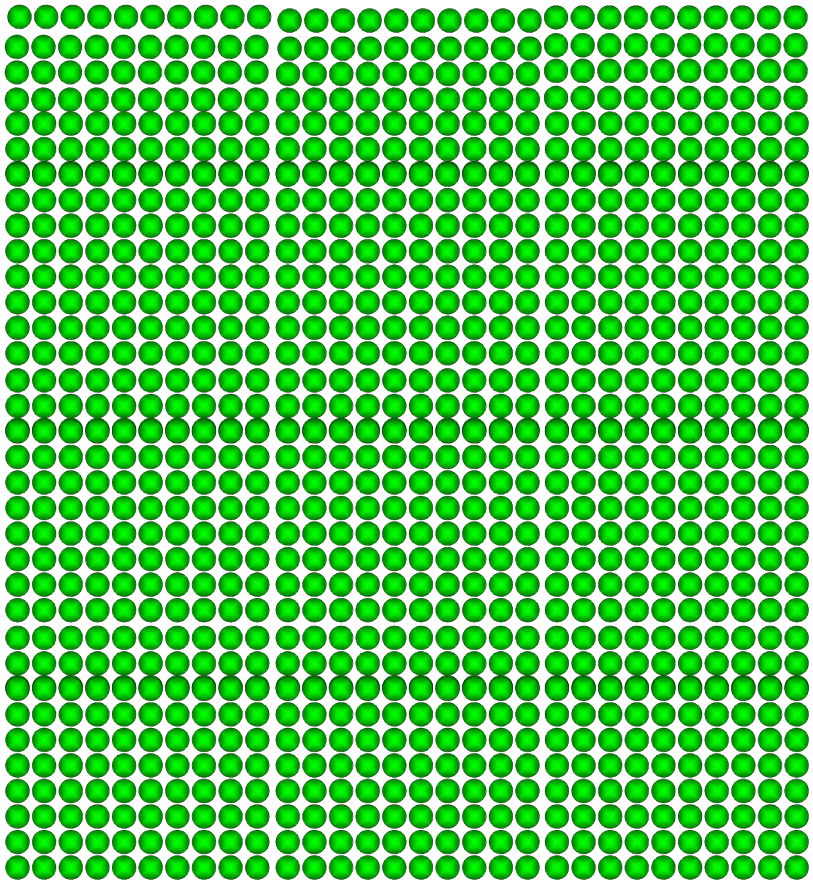
Scattering pattern of latex beads of 1µm in water in verticial polarization at 632,8 nm

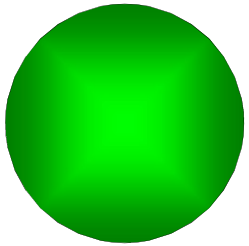
Distribution weighted by volume

1000 particles of 1 µm

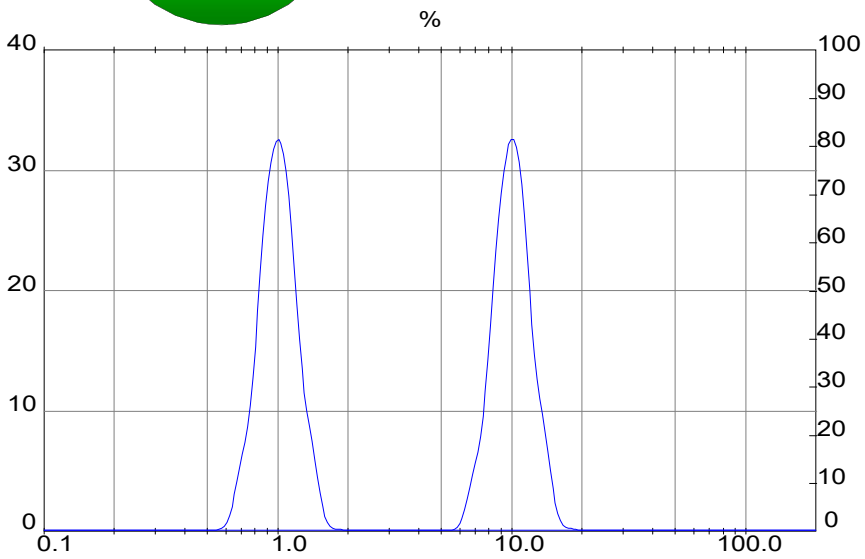
=

1 particle of 10 µm





Necessary: refractive index of particles and solvent !

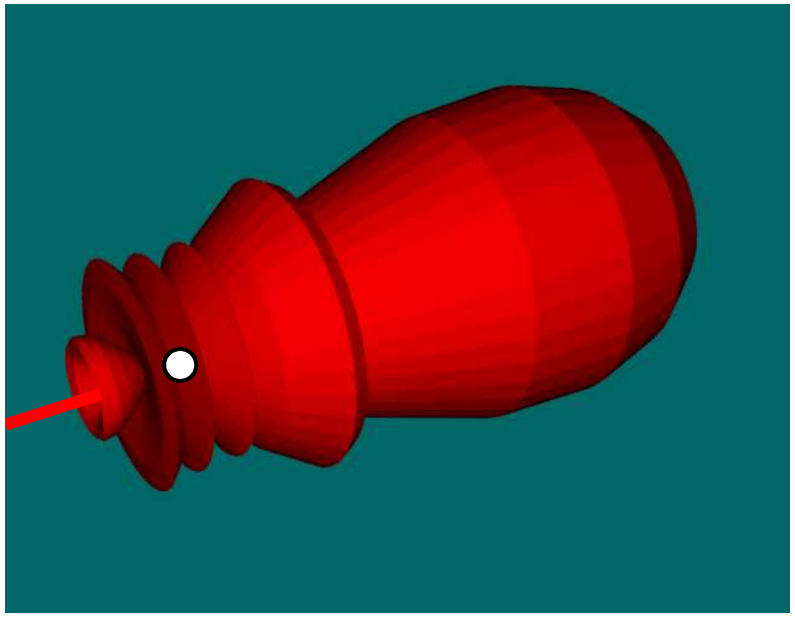


Volume Distribution of 1 particle of 10µm and 1000 particles of 1 µm.

Particle size analysers by Dynamic light scattering

Cells from 3 µl to 1 ml

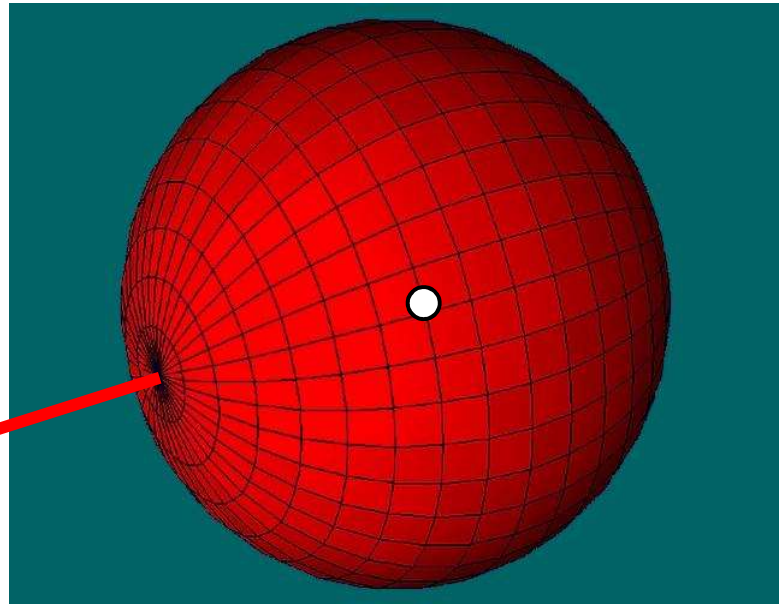
Laser Diffraction or DLS ?



$d > \lambda/10$ anisotropic diffusion of light, **diffraction** is possible based on Intensity=f(angle)

$d < \lambda/10$

isotropic diffusion of scattered light, DLS is recommanded Intensity=f(time)



Distributions weighted by intensity of scattered light

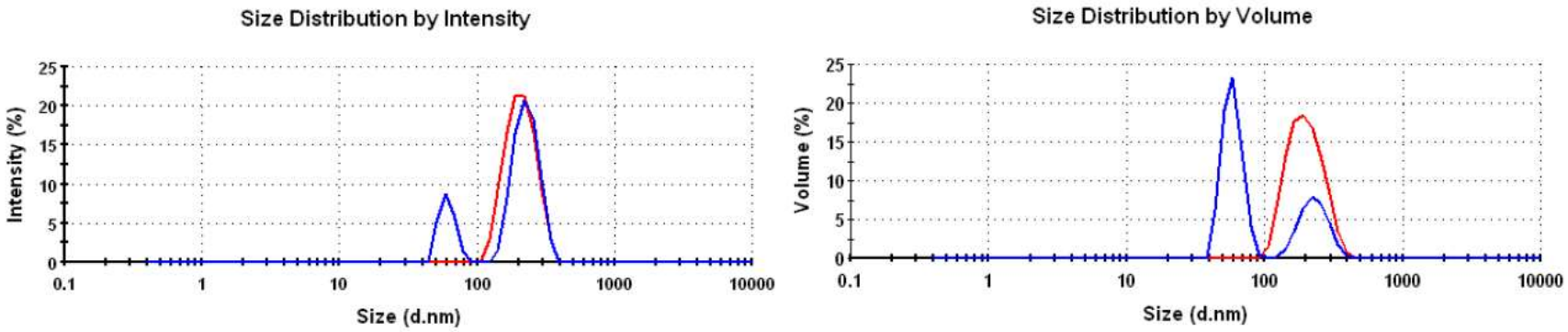
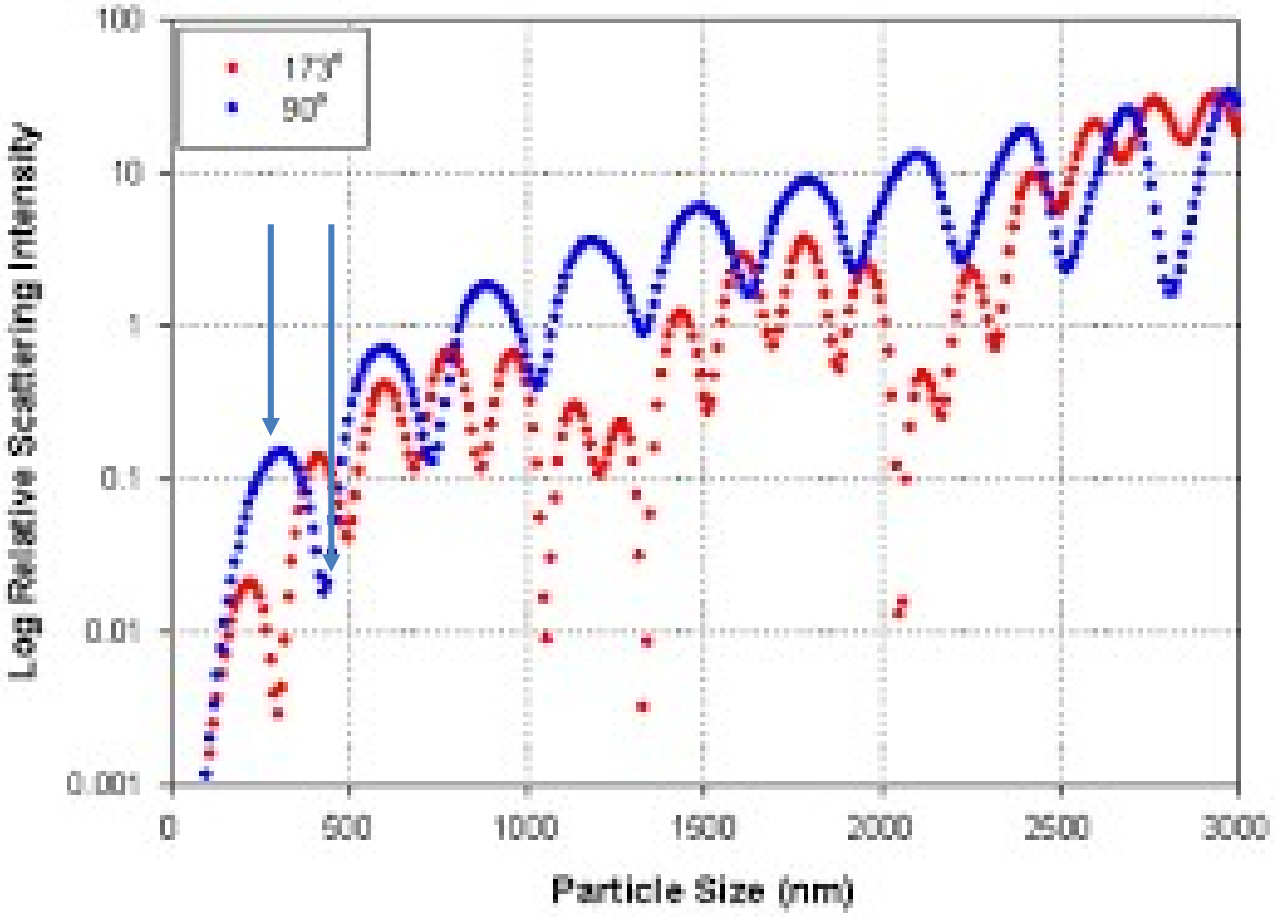


Figure 2: Intensity and volume size distributions for the 2.5:1 (60:220nm latex standard) volume mixture measured at 90° (**red**) and 173° (**blue**).

Result: You don't need refractive index of particles for intensity distribution and Z average and polydispersity calculation but refractive index of solvent is very important !

Hard to imagine volume distribution from intensity distribution

Scattered intensity vs size

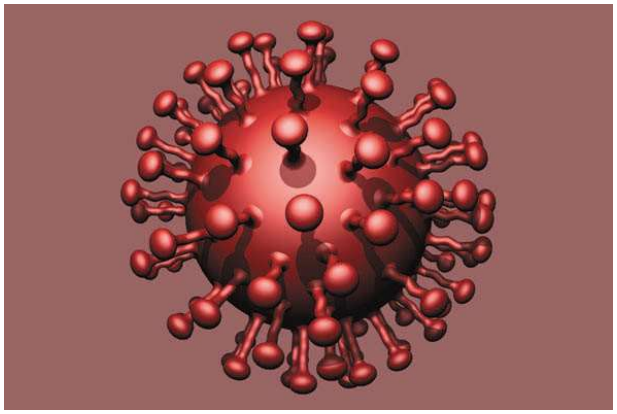


The intensity scattered by particles is not directly linear with size. Maximum and minimun scatterers exist for each angle.

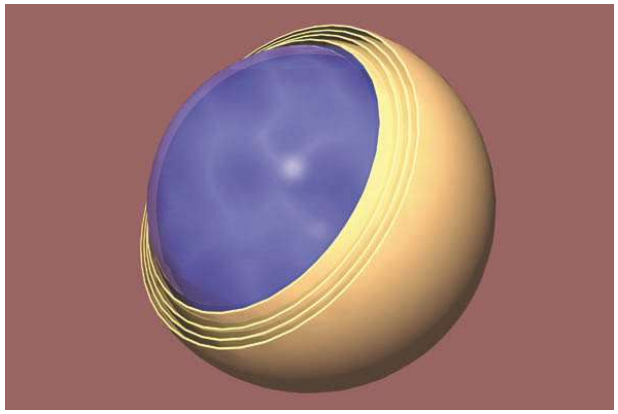
Ex: 300 nm scatters more light than 400nm at 90 degrees.

400 nm scatters more intensity than 500nm at 173 degrees.

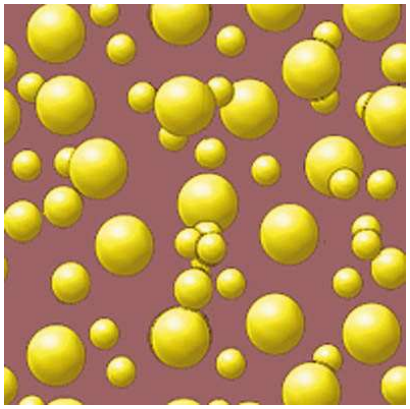
Applications



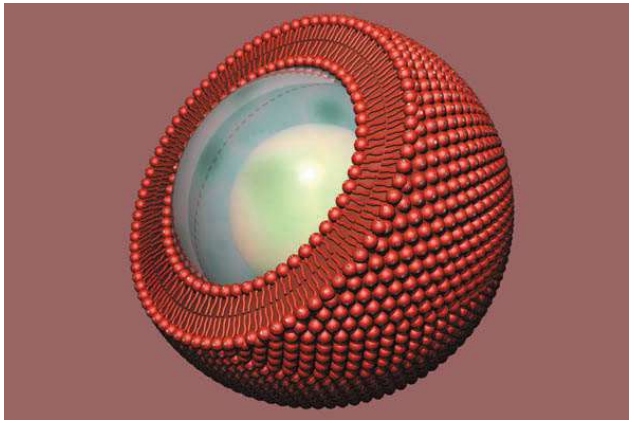
VIRUS



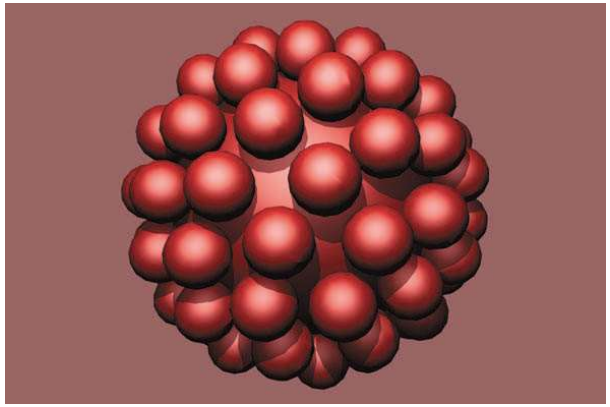
CAPSULE multilayers



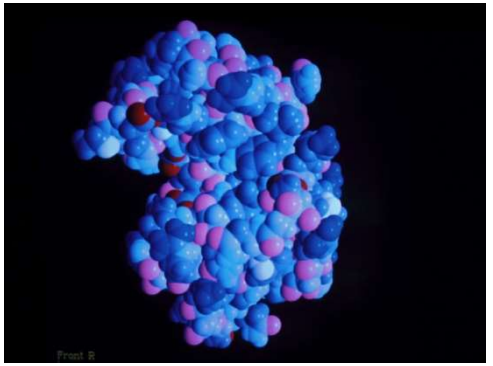
MICRO-EMULSION



LIPOSOME



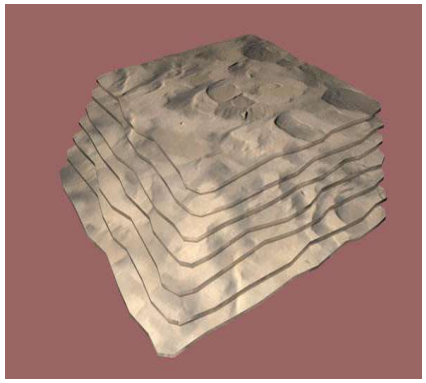
LATEX



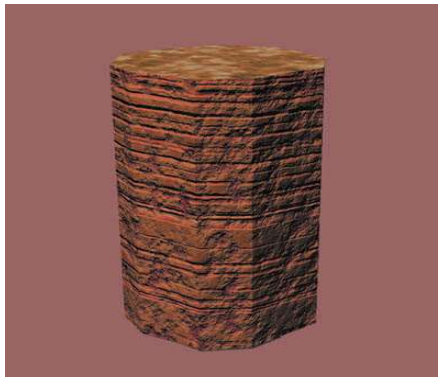
PROTEINE

+ ANTIBODY, MICELLES, VACCINeS, PEPTIDES, ENZYMES, HYDROGELS …

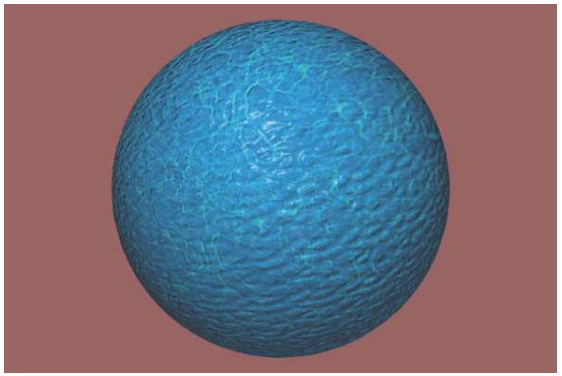
Applications



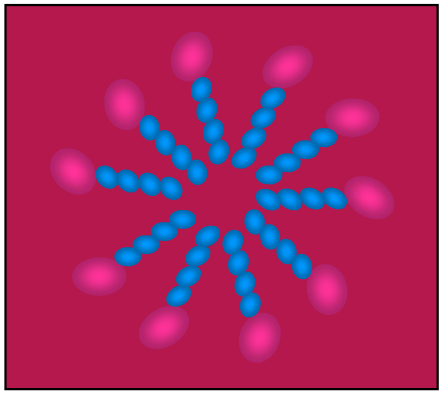
Ceramics



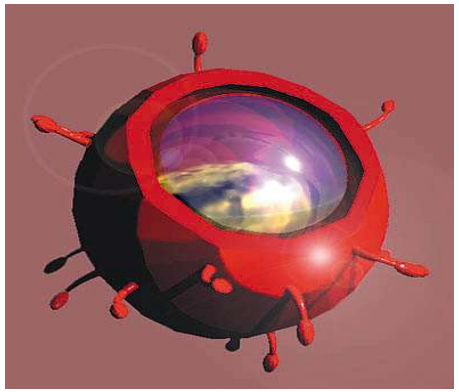
Metal Oxides



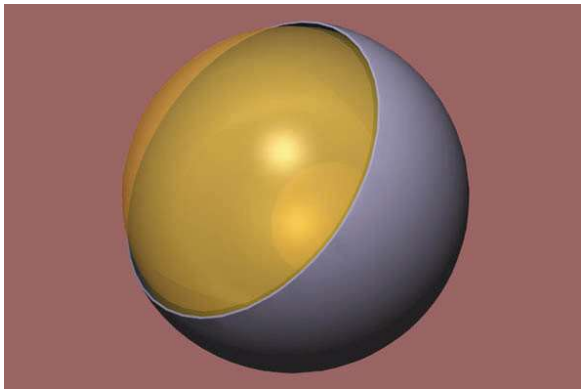
Silica



Micelles, MOFs



Polymers



NanoCapsule

Alumina, Inorganic particles or Mineral & organic pigments…

Size range vs technics

20 nm 50 nm Laser Diffraction 3500 µ

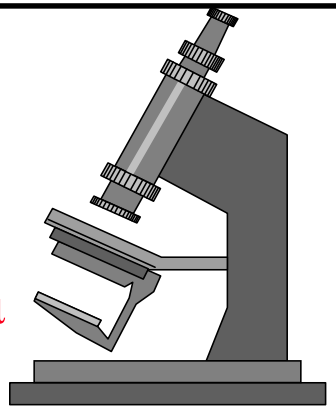
20 à 40µ Tamisage / Sieving several cm

6 Ä DLS (rétrodiffusion) 10 µ

Diffraction Rayons X

20 nm Sédimentation 100 µ

Disc centrifuge



0.5 µ Analyse d'image Plusieurs cm

Images analysis

0.5 µ Comptage particulaire x00 µ

Counters

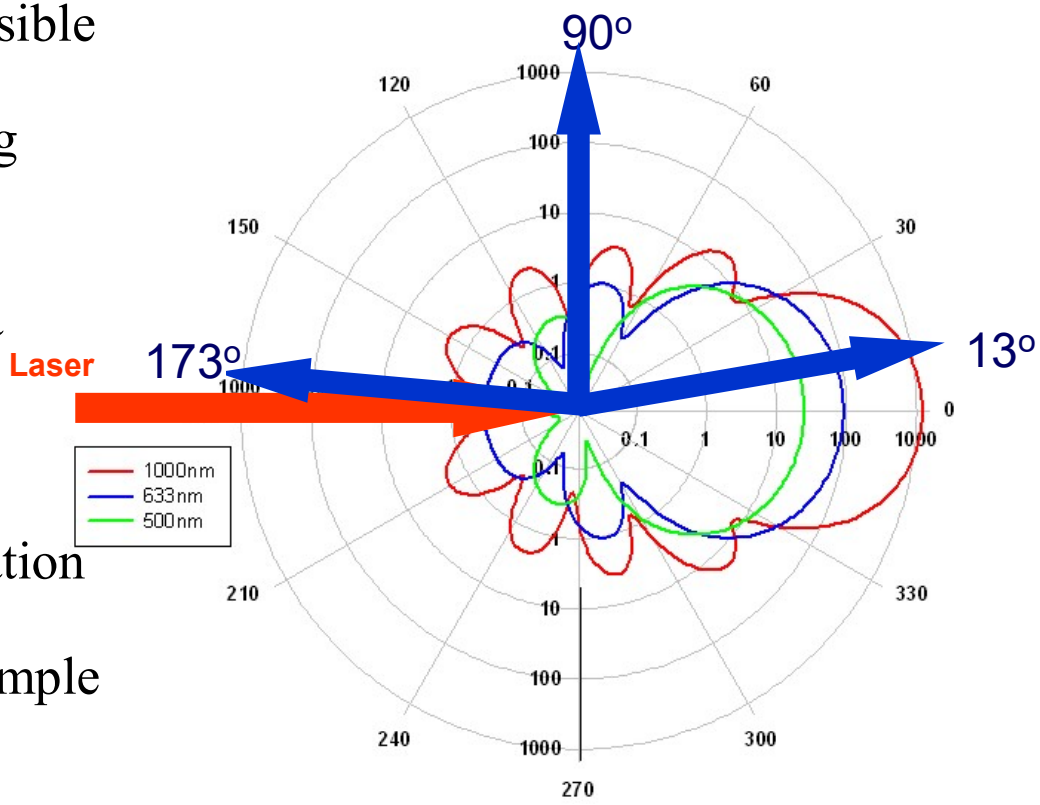
DLS versus angles

- Mesure en rétrodiffusion: moins sensible aux grosses particules
- Backscattering is less sensitive to big particles

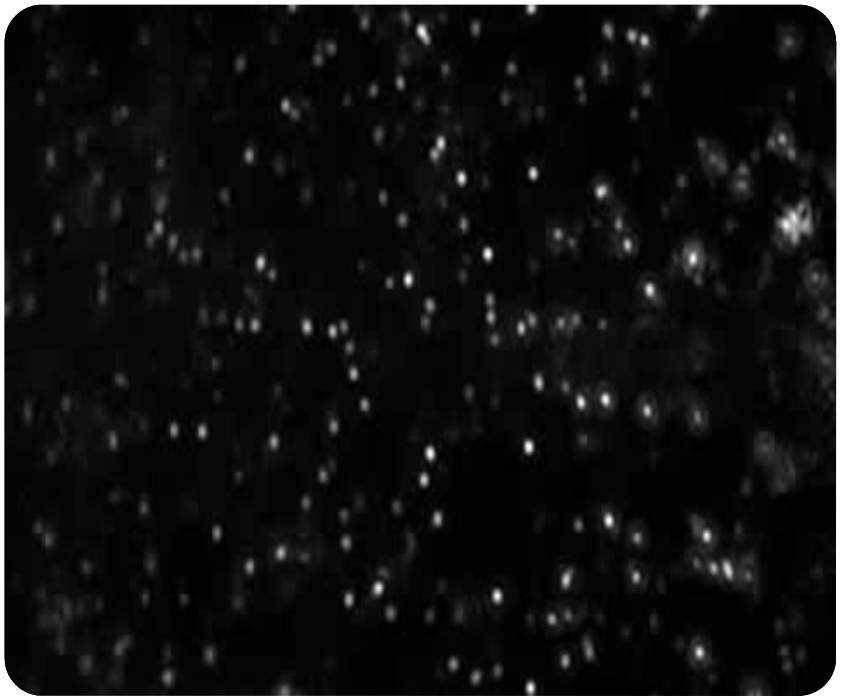
- Rétrodiffusion plus robuste contre la poussière et les grosses particules
- Backscattering less sensitive to dust

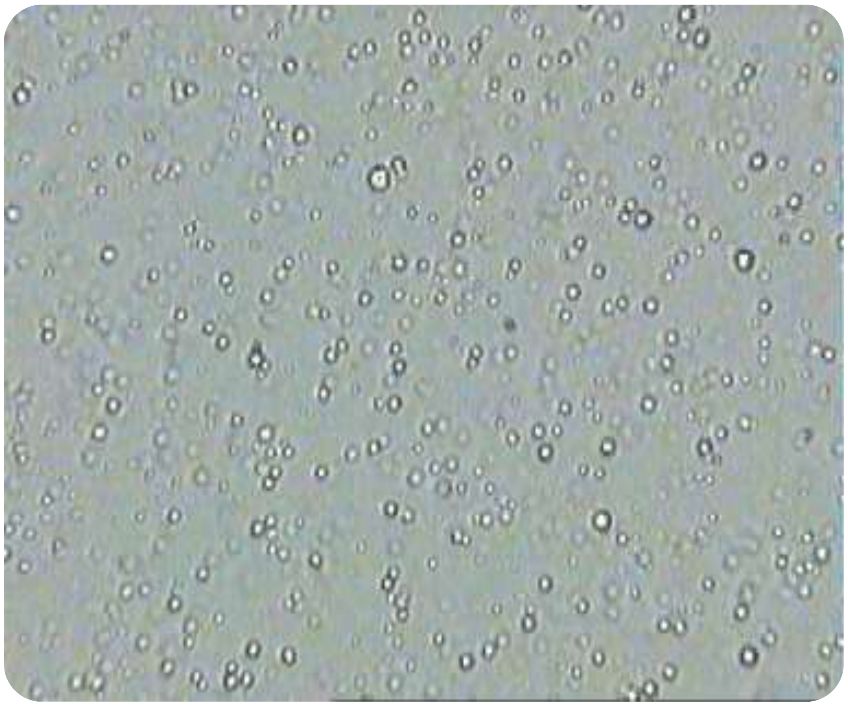
- Reduit les biais associés à la préparation d'échantillon
- Backscattering is less sensitive to sample prep

- Meilleure reproductibilité
- Better reproducibility of results



Brownian Motion

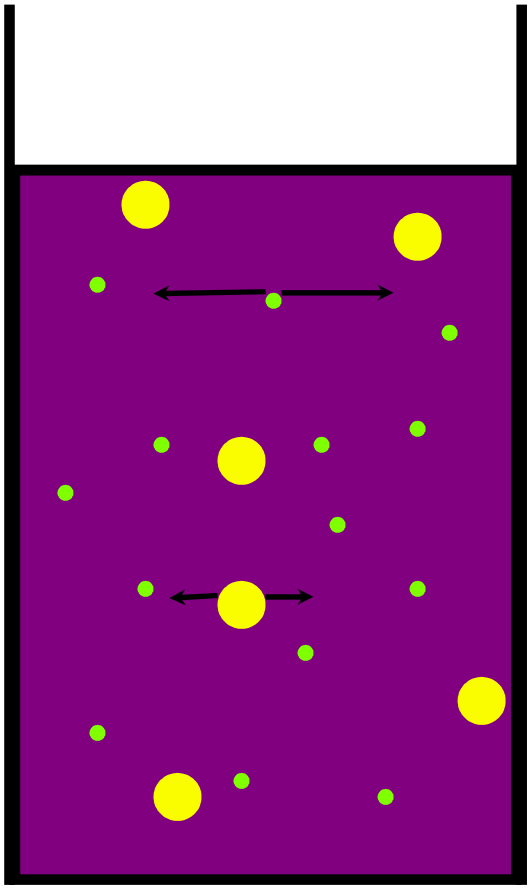


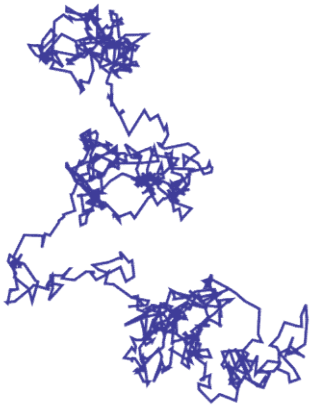


Small Particle = Strong Diffusion Coefficient = Quick intensity fluctuations
Big Particle = Weak Diffusion Coefficient = Slow intensity fluctuations

DLS measures intensity fluctuations of scattered light, created by Brownian motion of diffusing particles.

Definition of Brownian motion





Discovered in 1827 by Robert BROWN:
«particles in suspension are moved by shocks with small molecules»

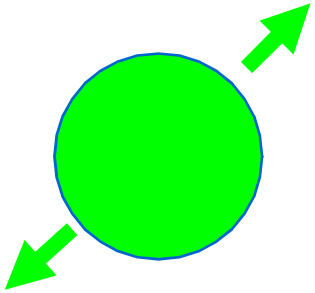
D (m^2/s): translational diffusion coefficient

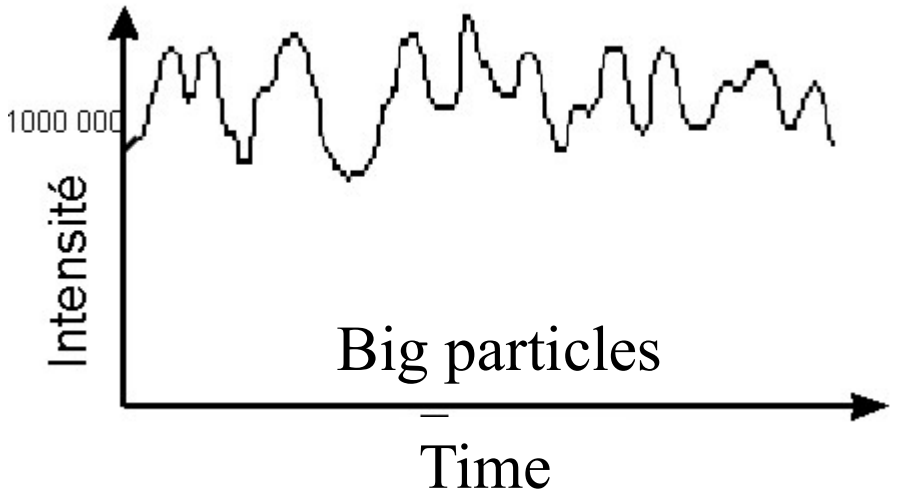
$X^2 = 2 D t$

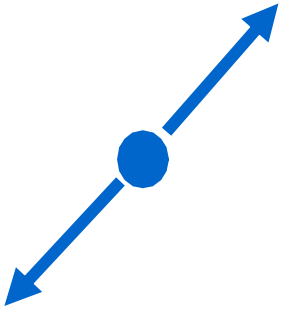
X^2 = mean square displacement
t = time
X = mean displacement of the particles

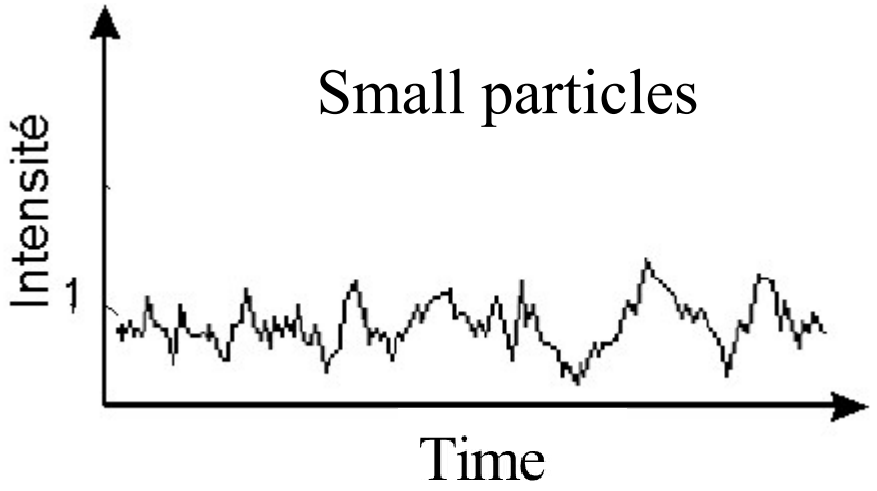
X should vary as the square root of time which characterizes brownian motion
Be careful with thermal fluctuation while you heat the sample by the bottom with Peltier effect and with sedimentation if the density of particles is high

intensity fluctuations vs size

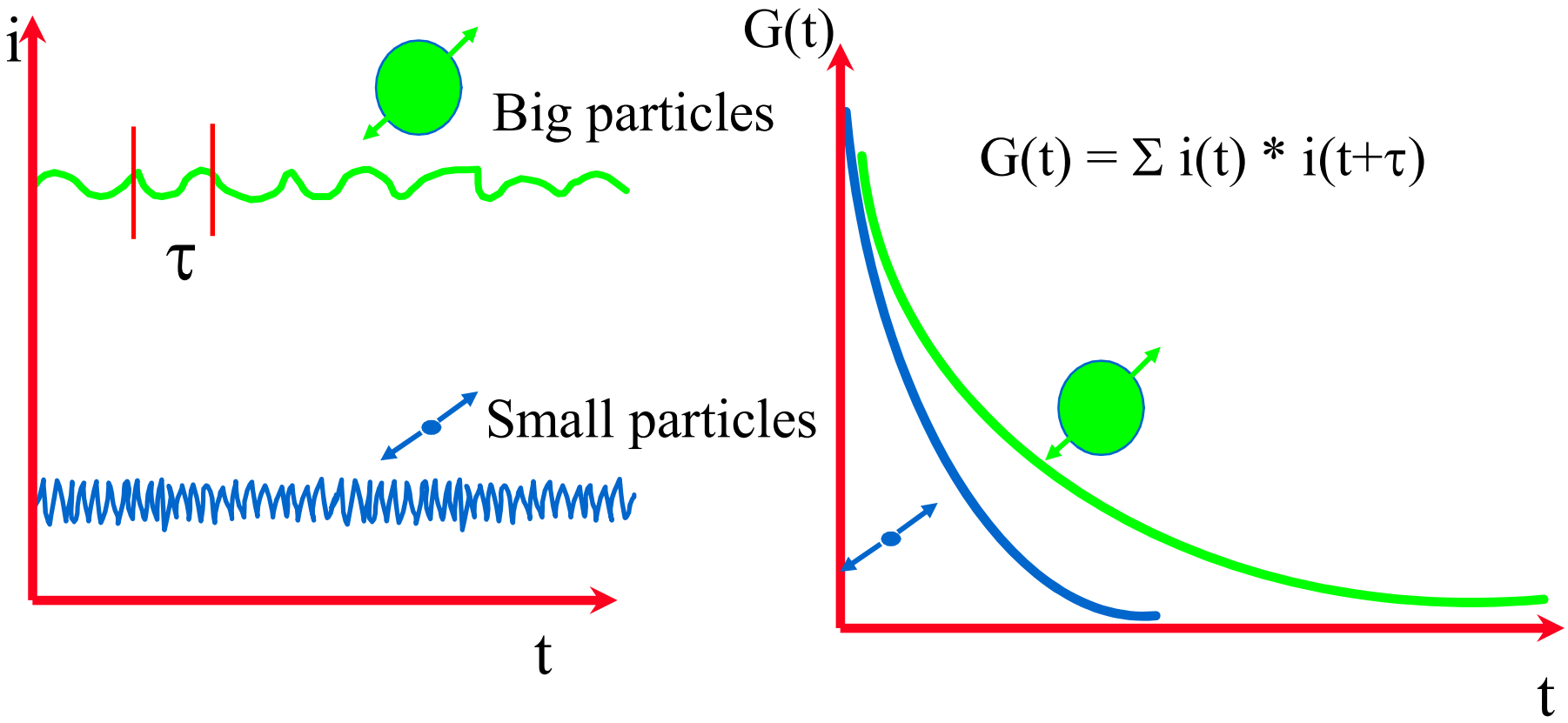






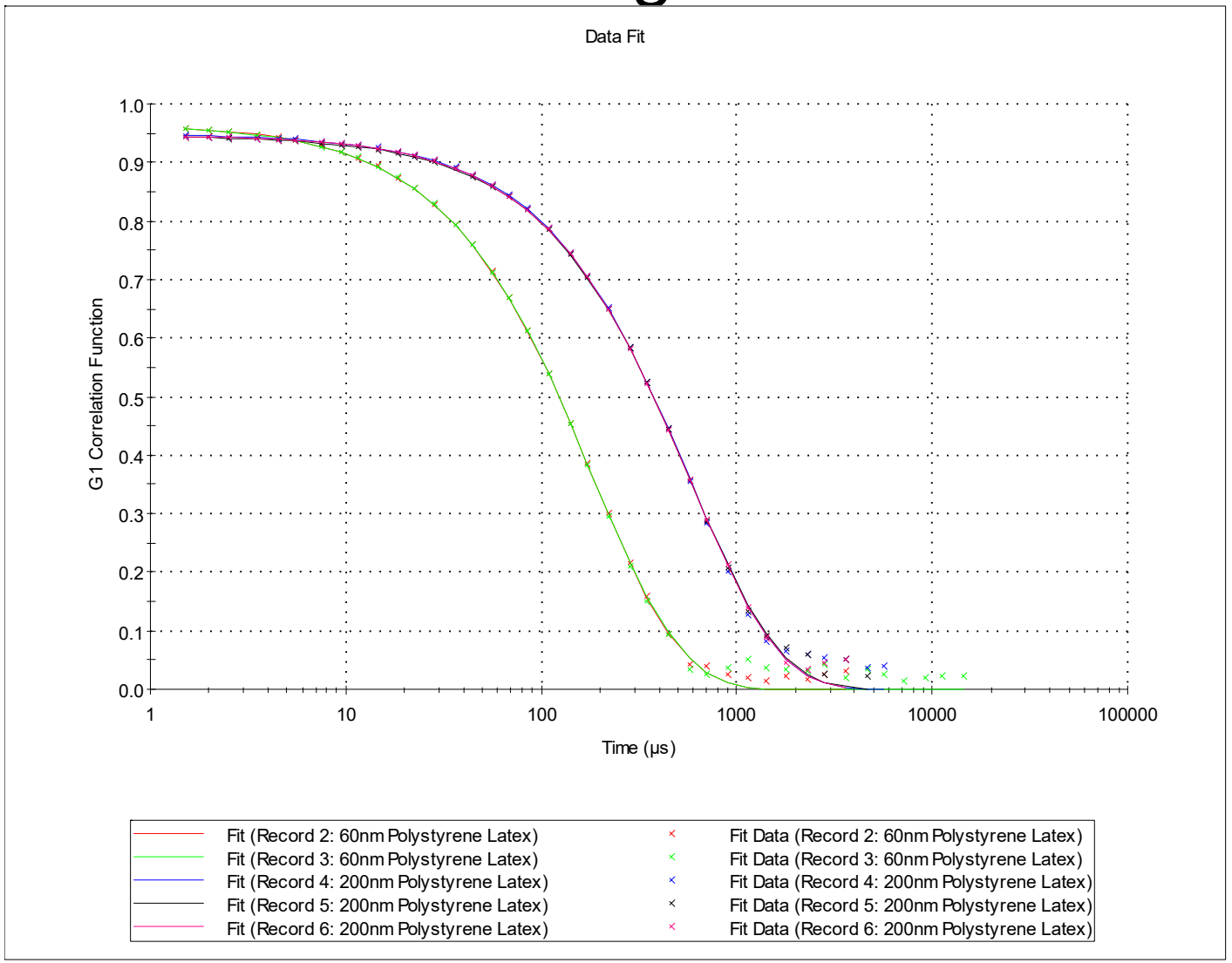


Autocorrelation function



τ sampling time of correlator from μs to ms

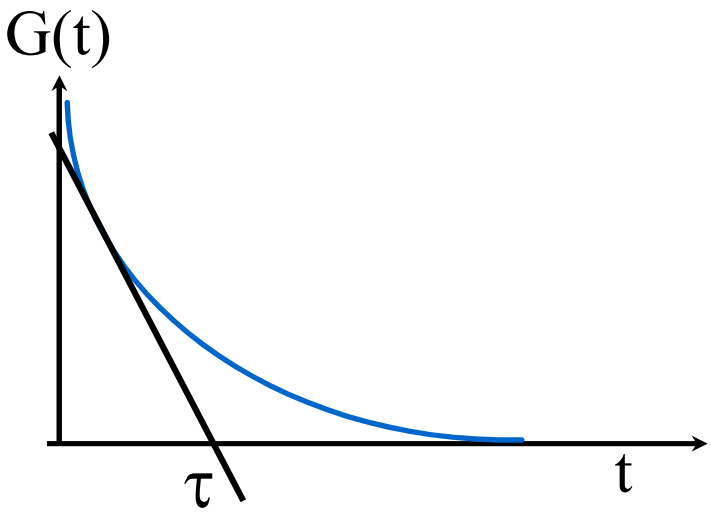
Correlation function displayed in linear mode than in log mode.



Auto-correlation function analysis

For a single size distribution

$$G(\tau) = \exp(-\Gamma . \tau) = \exp(-D_0 q^2 t)$$

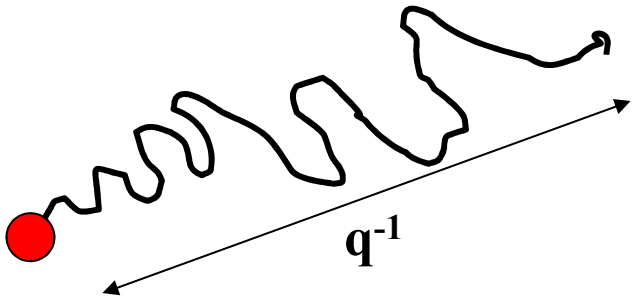


τ = sampling time of correlator

$\Gamma = D_0 q^2$ = decreasing rate

D_0 =translational diffusion coefficient

q = wave vector $= \frac{4\pi n}{\lambda} \left(\sin \frac{\theta}{2}\right)$



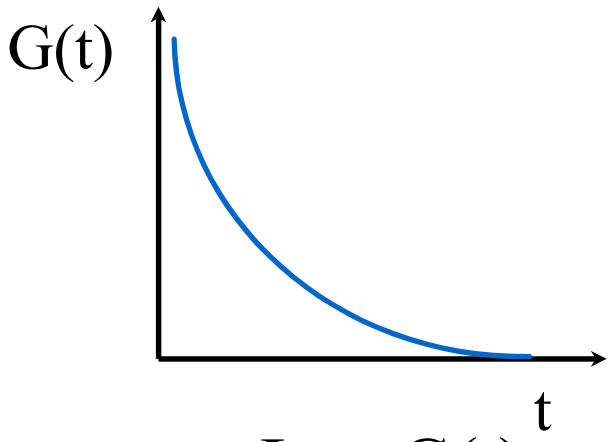
n = refractive index of suspension medium

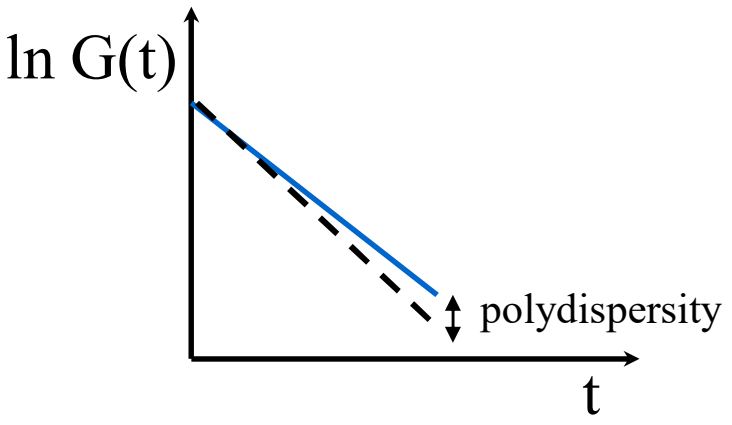
λ = wavelength of laser

θ = angle between laser and photomultiplier

q^{-1} = when particle moves **q^{-1}** she has lost position correlation (observation scale)

1. Cumulants analysis (monomodal – ISO 13321)





$$\text{Log } G(t) = a + bt + ct^2$$

$b = 1/\tau = Dq^2$ ➔ we know q we calculate D translational diffusion coefficient

$2c / b^2$ = Polydispersity (standard deviation of the size distribution)

Hydrodynamic diameter

STOKES-EINSTEIN law

$$d_H = \frac{k\,T}{3\,\pi\,\eta\,D}$$

EINSTEIN:
Thermal energy
of particle

NEWTON:
Viscous drag
of particle

d_H : hydrodynamic diameter (nm)

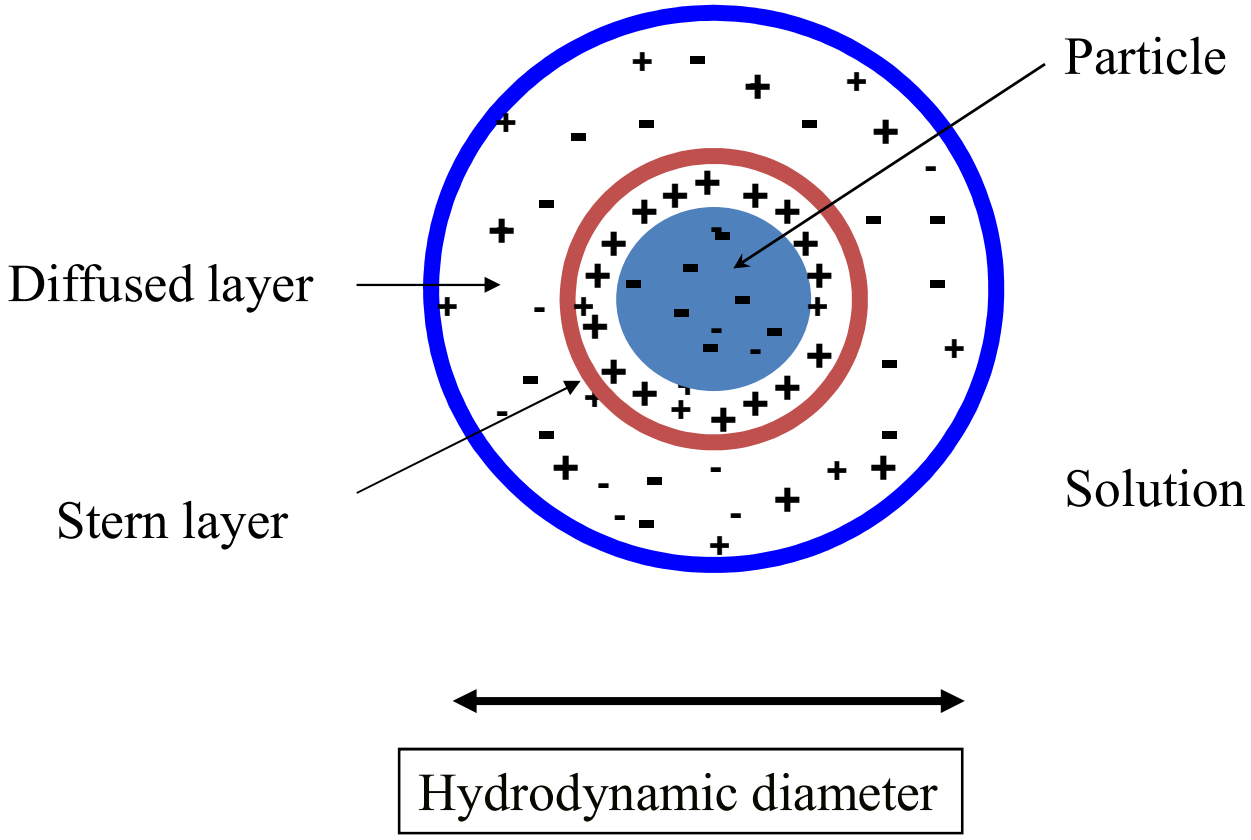
T : absolute temperature

D : translational diffusion coefficient

η : Dynamic viscosity (cP) of dilution medium (water)

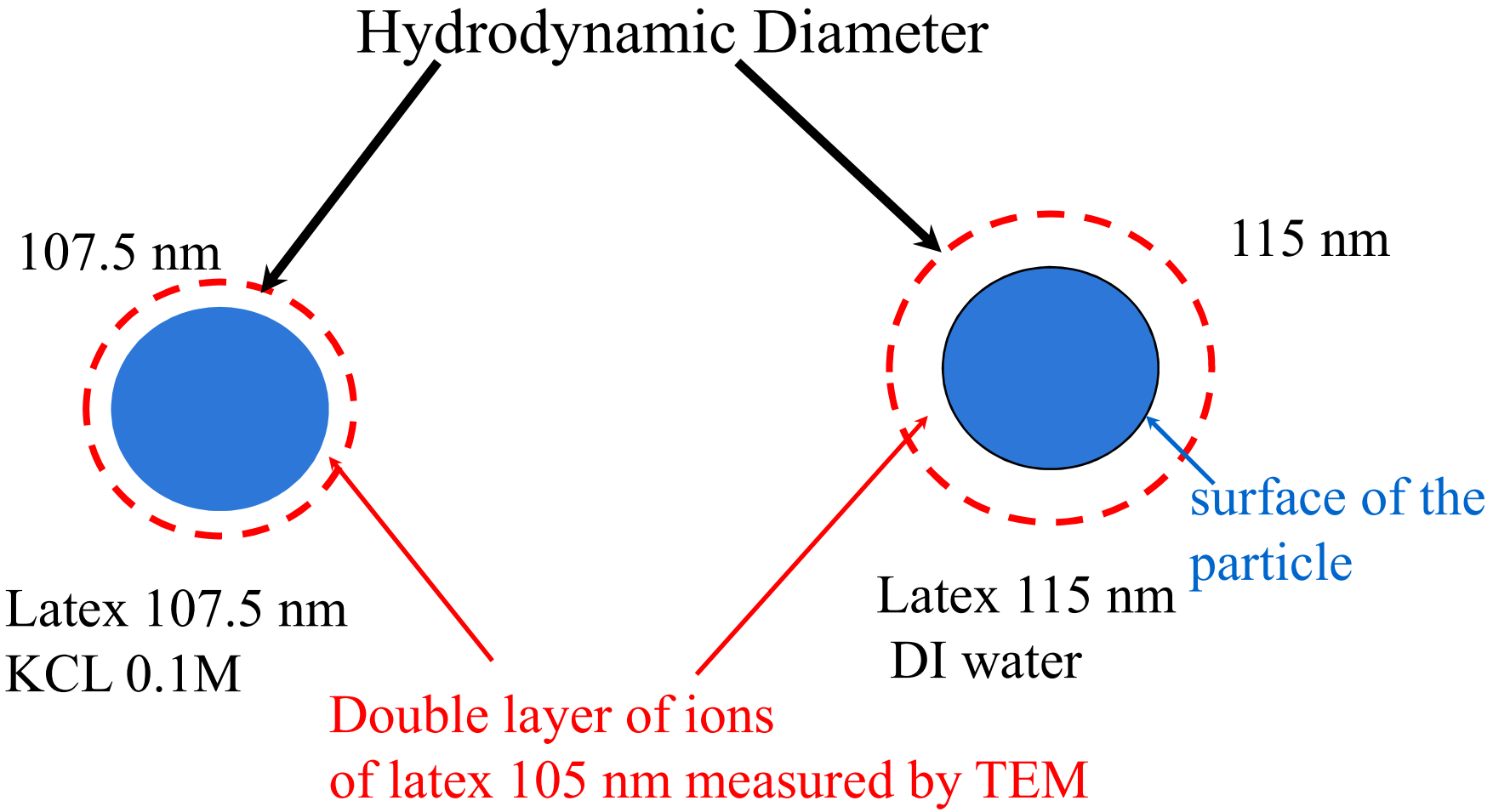
k : Boltzmann constant

Meaning of hydrodynamic diameter



The hydro-dynamic diameter represents the size of the particle + the thickness of the diffused layer around the particle.

Example of hydrodynamic diameters

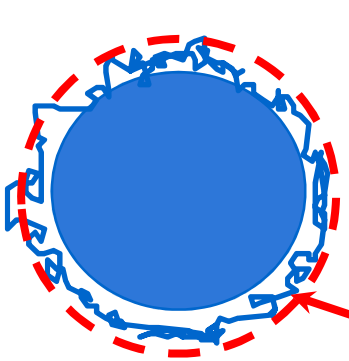


Thickness of the double layer

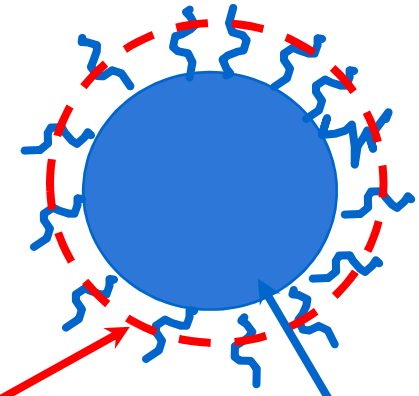
Depending on salt concentration and valency of ions, double layer goes from 1 nm to 1000 nm!

CONC (MoI/L)	ELECTROLYTE					
	1:1	1:2,2:1	2:2	1:3,3:1	3:3	2:3,3:2
10^{-1}	0.96	0.55	0.48	0.39	0.32	0.25
10^{-2}	3.04	1.76	1.52	1.24	1.02	0.78
10^{-3}	9.61	5.55	4.81	3.92	3.20	2.48
10^{-4}	30.4	17.6	15.2	12.4	10.2	7.85
10^{-5}	96.1	55.5	48.1	39.2	32.0	24.8
10^{-6}	304	176	152	124	102	78.5
10^{-7}	961	555	481	392	320	248

Example of hydrodynamic diameter

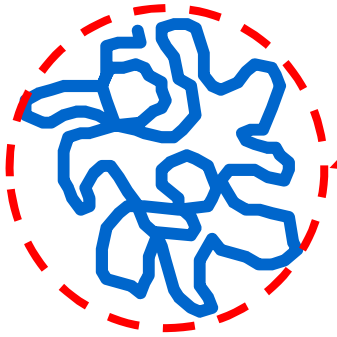


Particle solid +
Adsorbed Polymer



Hydrodynamic diameter

surface of
the particle

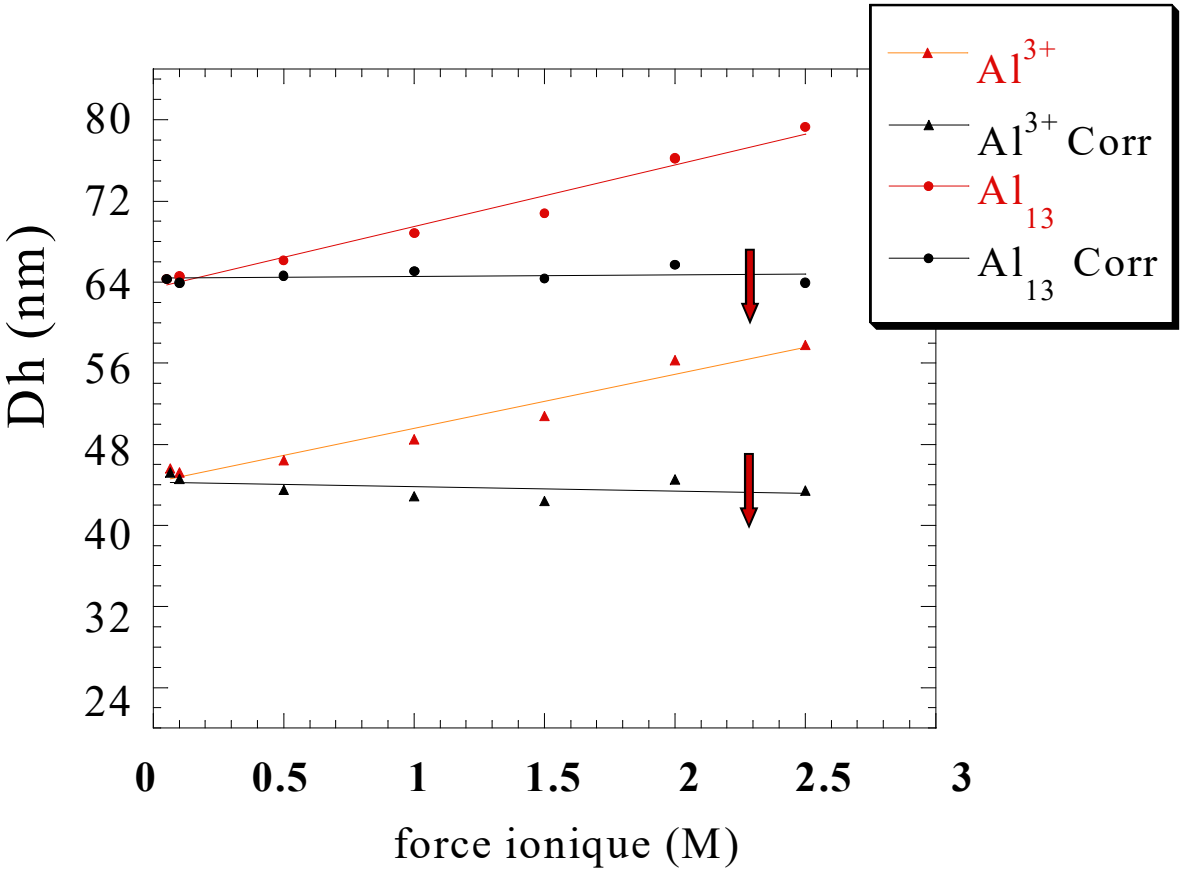


Polymer

Case of non spherical particles:
d(H) is the diameter of a sphere
having the same diffusion coefficient (CNT,
platelike particles, Nanorods, Needles)

Radius of gyration can be a better geometric descriptor in this case

Don’t forget to enter ionic force before calculation of Dh



Be careful :
viscosity changes with salt concentration !

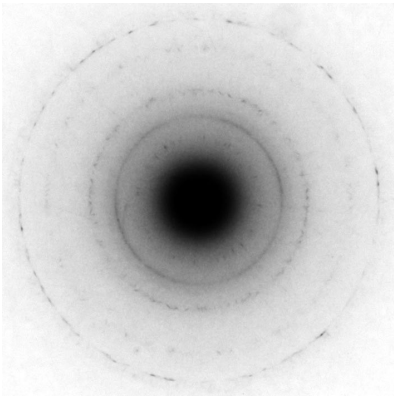
$$Dh = kT/3\pi\eta D$$

D: diffusion coefficient
k : Boltzmann constant
T: temperature
η: viscosity

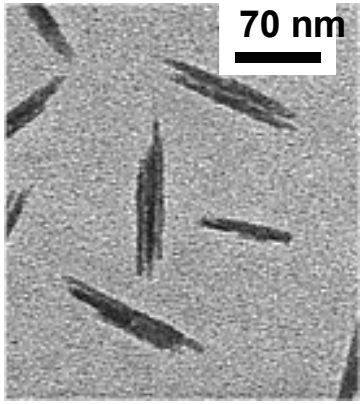
Dh =f(ionic strength) = constant

Particles are stable with dilution, if we enter ionic force

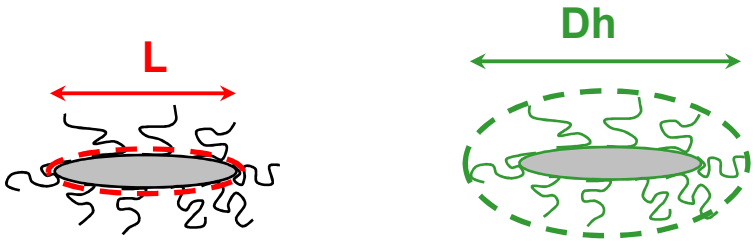
Main example! : particles with mineral core and polymeric shell



particles cristals



Where is the polymer ?



Polymer	TEM Length (nm)	Dh (nm)
10000	85	**102**
30000	70	**140**
60000	85	**334**

Dh increase when the molecular weight of the copolymer increases

The size of the crystal (TEM) is ~ constant:

Copolymer is grafted around the crystal.

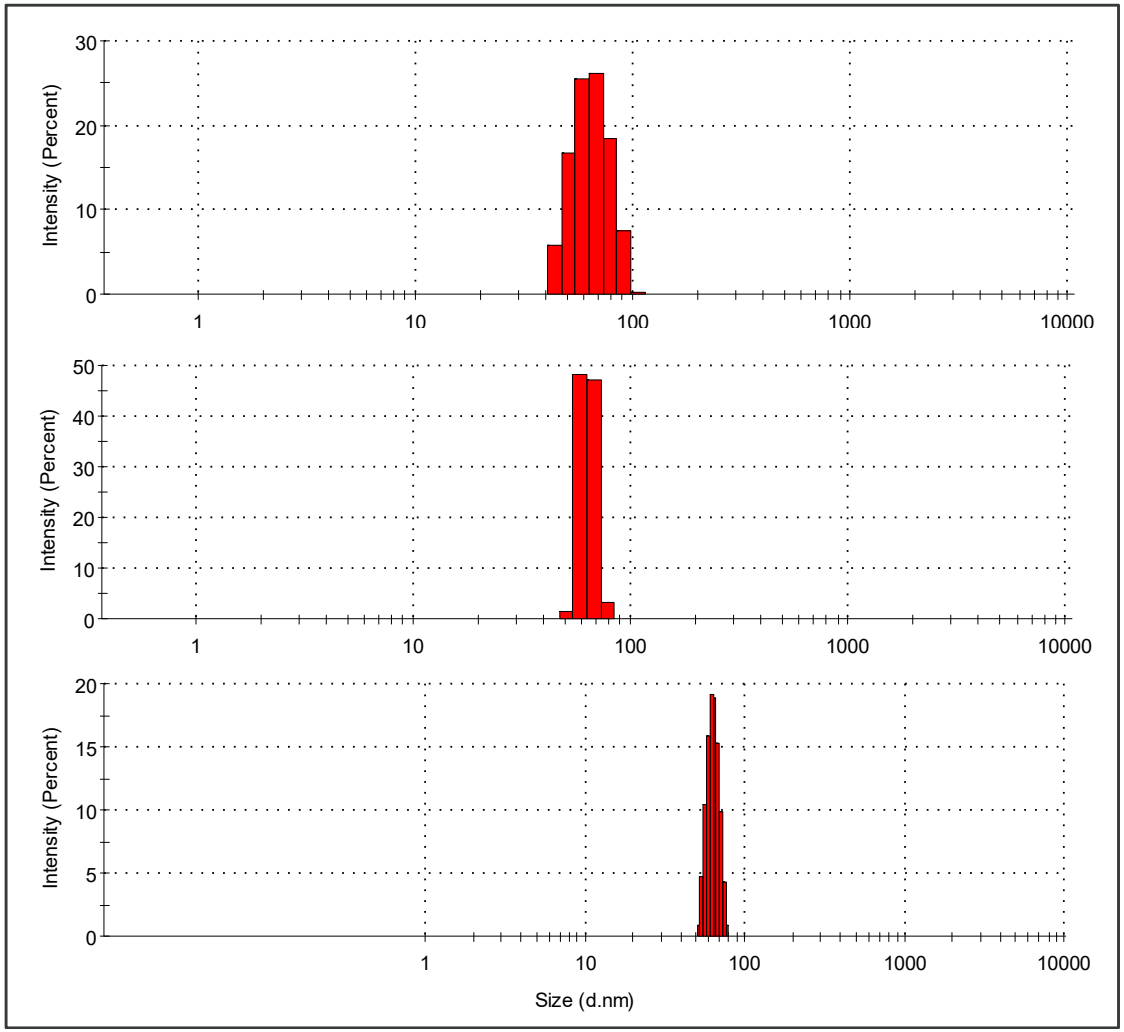
Let’s talk about increasing resolution of DLS result

Standard resolution
(70 classes)

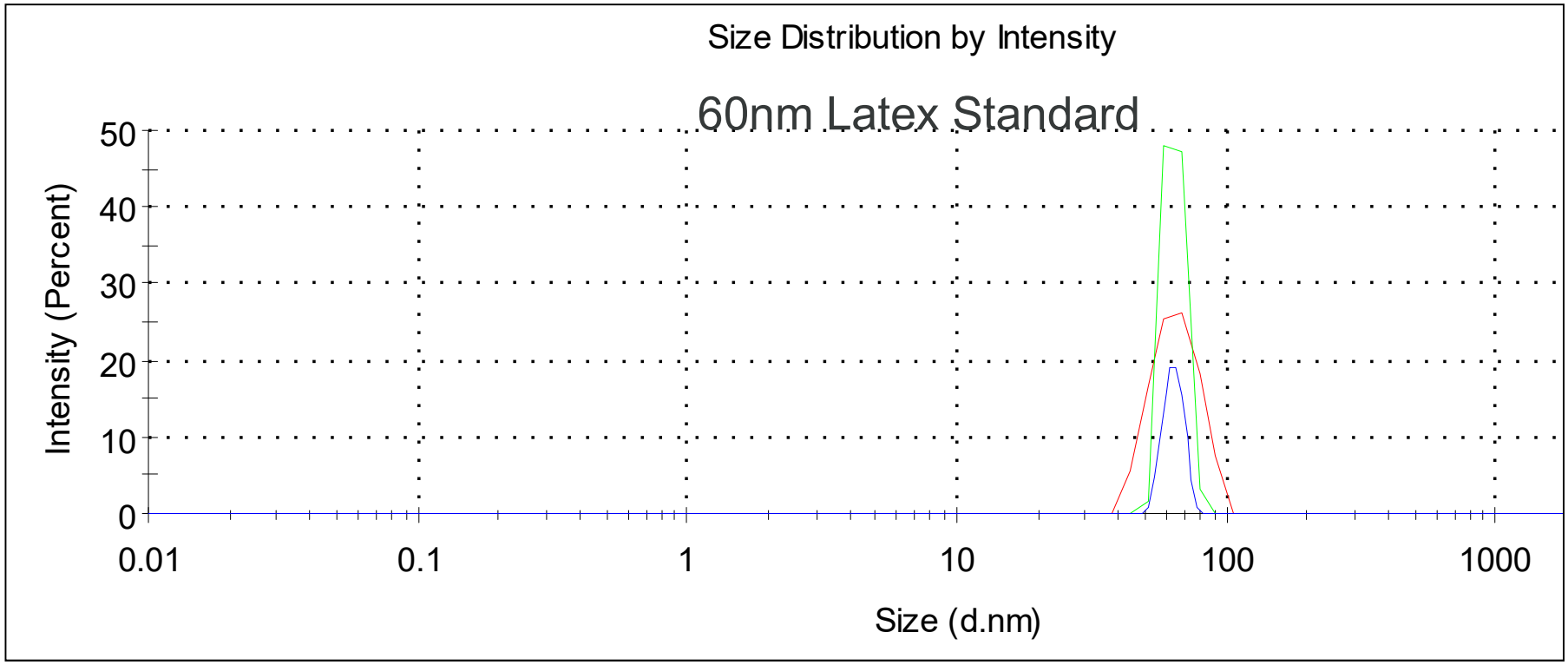
High resolution
(70 classes)

Very high resolution
(300 classes)

60nm Latex Standard:



Be careful interpreting DLS intensity result



General low resolution analysis

High resolution analysis

Very high resolution analysis

Never compare laser diffraction result with DLS until you convert Intensity DLS in volume distribution.

Never compare DLS intensity particle size with microscopy until you convert Intensity DLS in number distribution like microscope (TEM or SEM).

Sizing molecule correctly

Red : high resolution for a correct size distribution
Green : normal resolution for a distribution width, unrealistic for this molecule

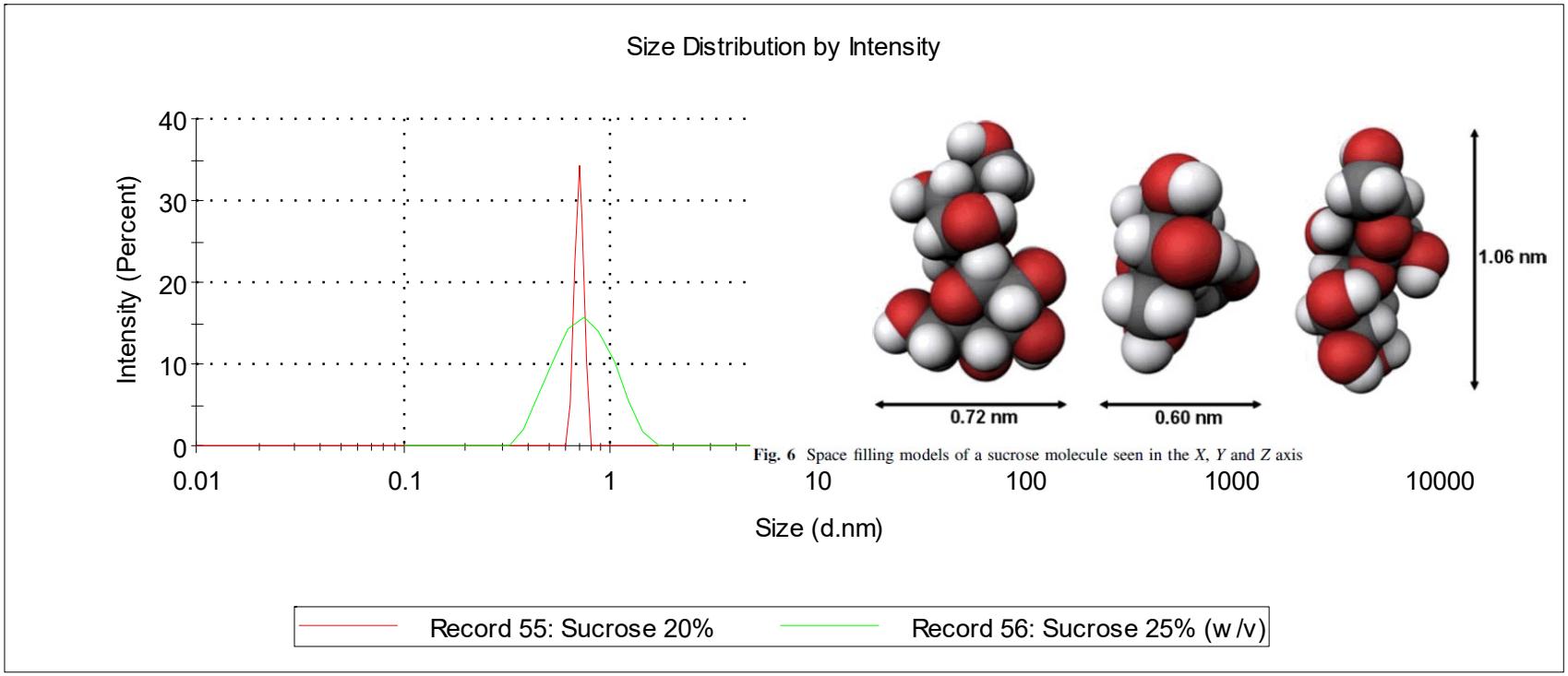


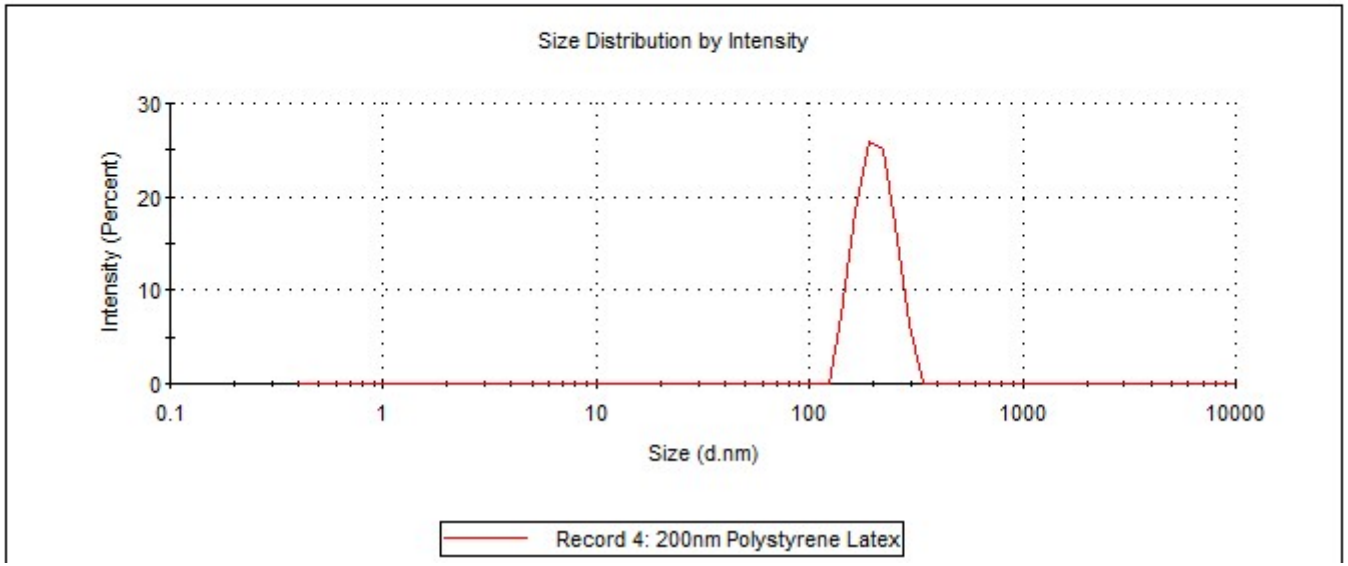
Fig. 6 Space filling models of a sucrose molecule seen in the *X*, *Y* and *Z* axis

After high resolution calculation, if you convert intensity distribution in number distribution, the peak remains at the right position (0.7 nm) and do not shift towards 0.3 nm.

Mathematical verification by Jean Christophe Gimel (Angers university)

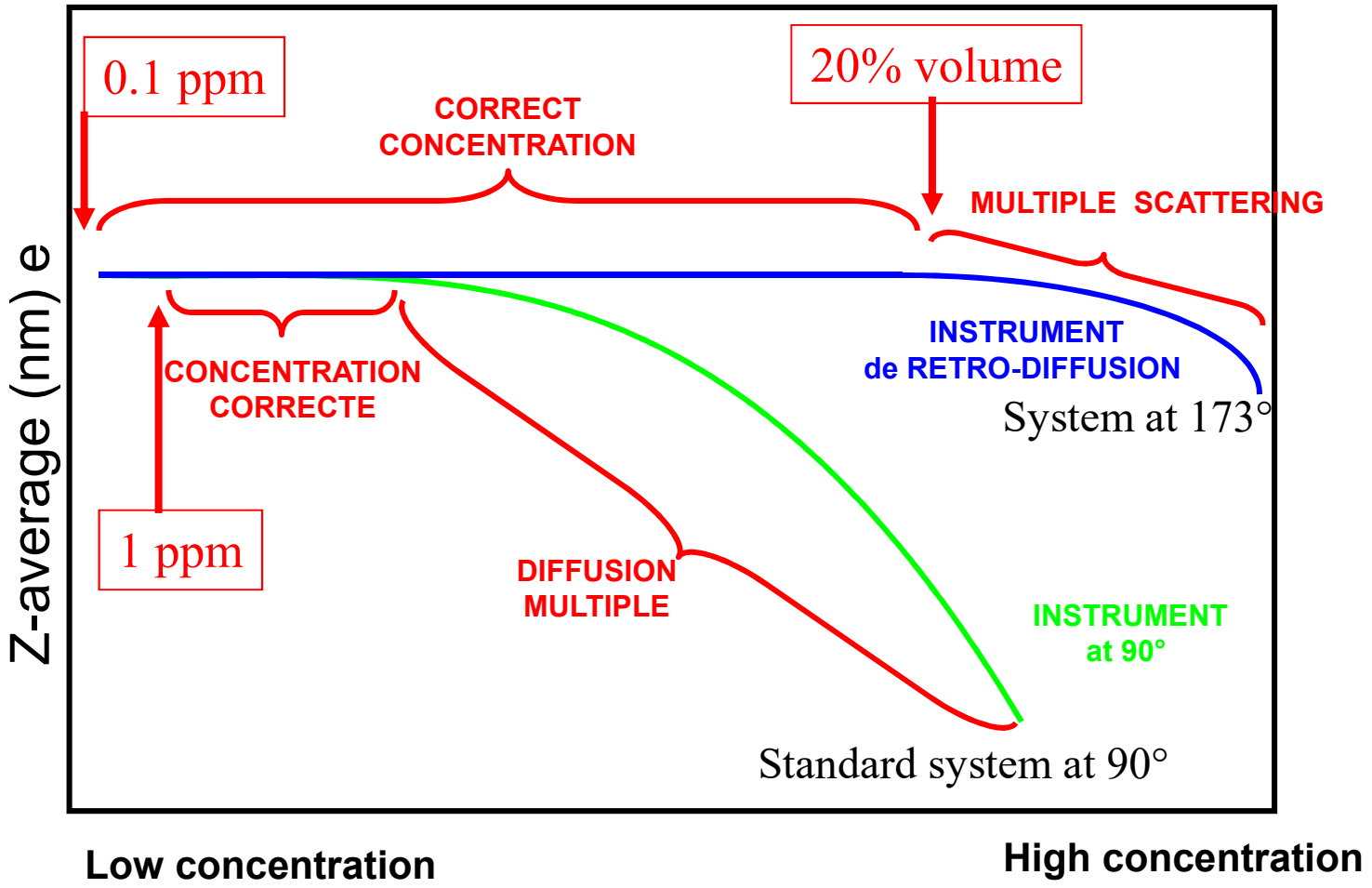
- If you divide the peak width (standard deviation) by the Z average (d_int) and you apply a square of this value: (sd/d_int)^2.
- For a log-normal distribution, you get a value close to the PdI.
- $(40{,}67 / 207)^2 = 0{,}038$ is close to PdI =0,030 (cumulant analysis)

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 202,0	Peak 1:	207,0	100,0	40,67
PdI: 0,030	Peak 2:	0,000	0,0	0,000
Intercept: 0,948	Peak 3:	0,000	0,0	0,000
Result quality : Good				



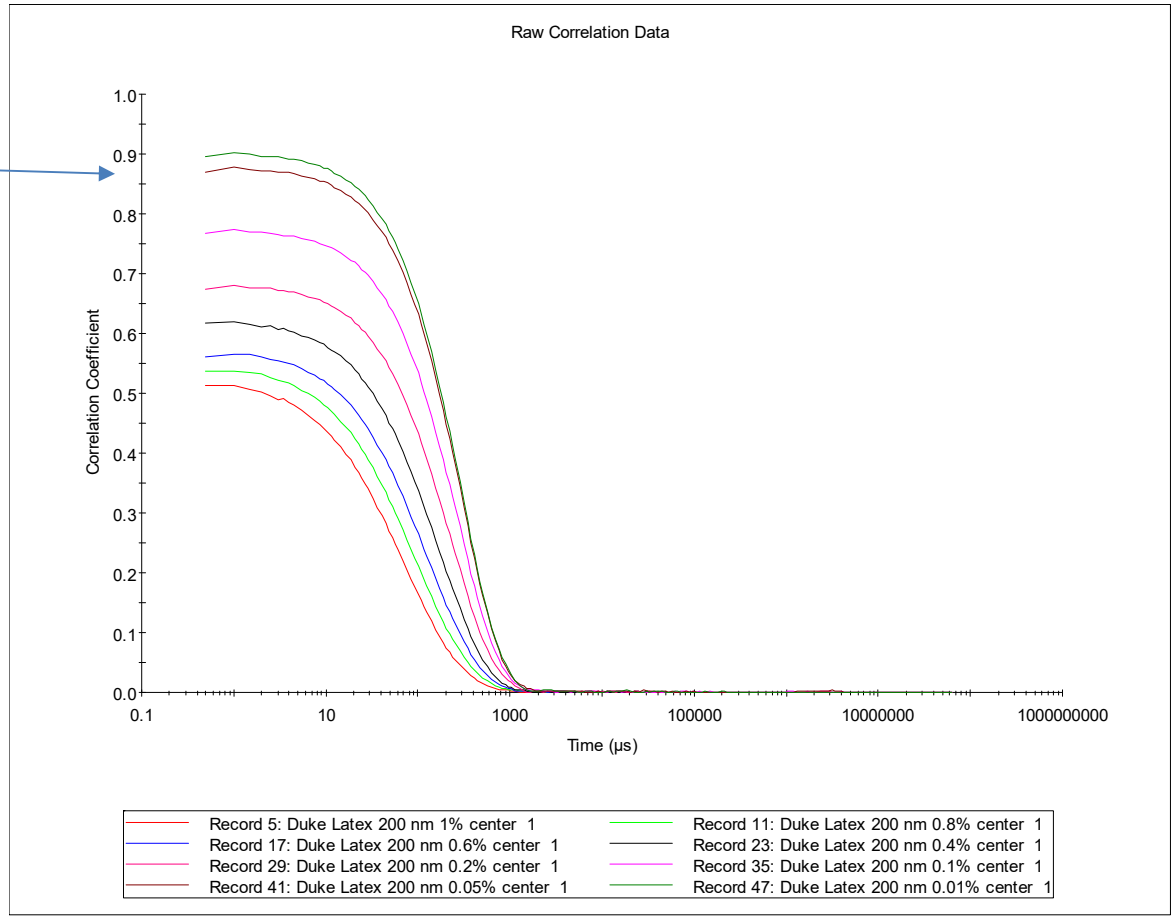
Avoiding multiple diffusion

- ISO standards require measurement on the plateau before multiple scatering starts when the concentration is too high



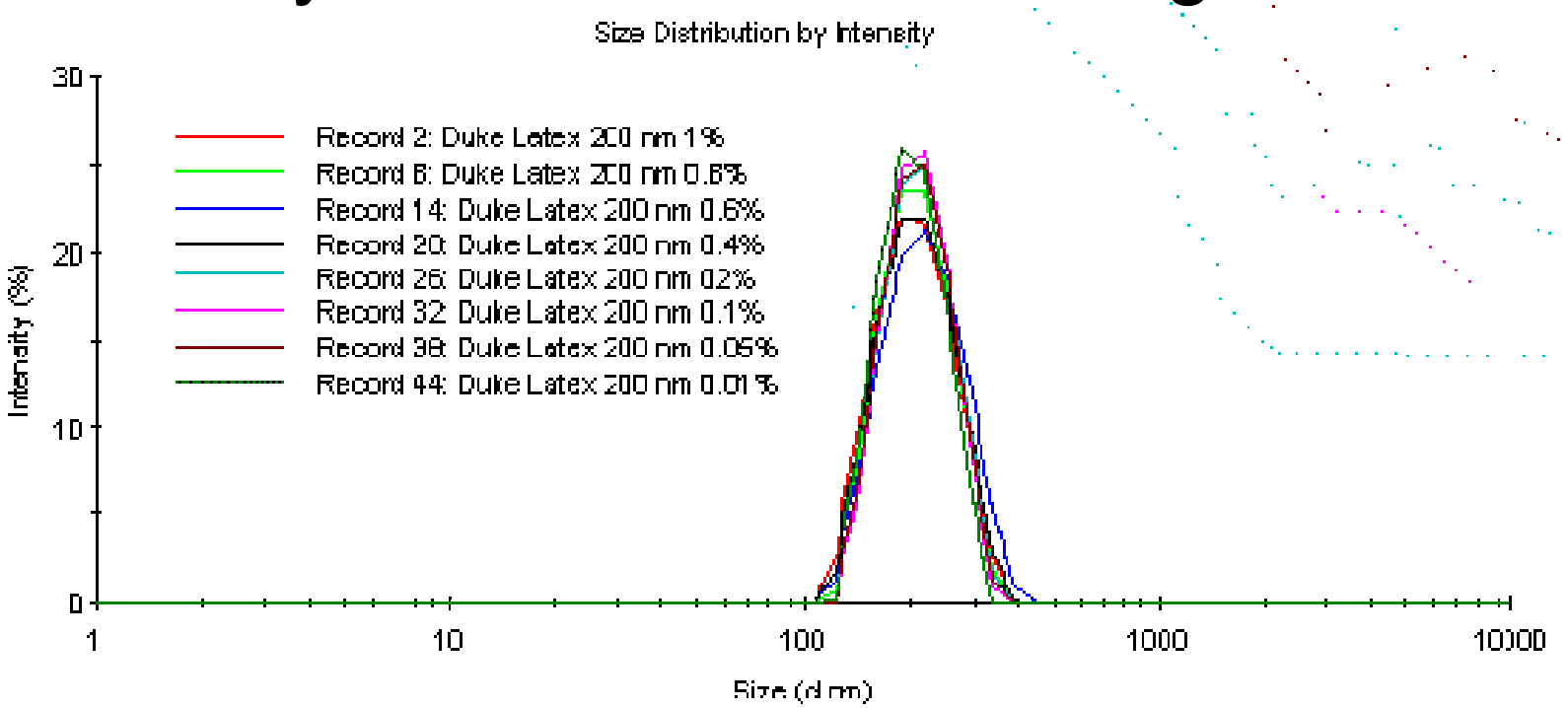
Correlogram with multiple scattering

Y



- The amplitude in Y (also called intercept) of the correlation function decreases when the concentration increases.
- When intercept is maximum, multiple scattering is minimum…

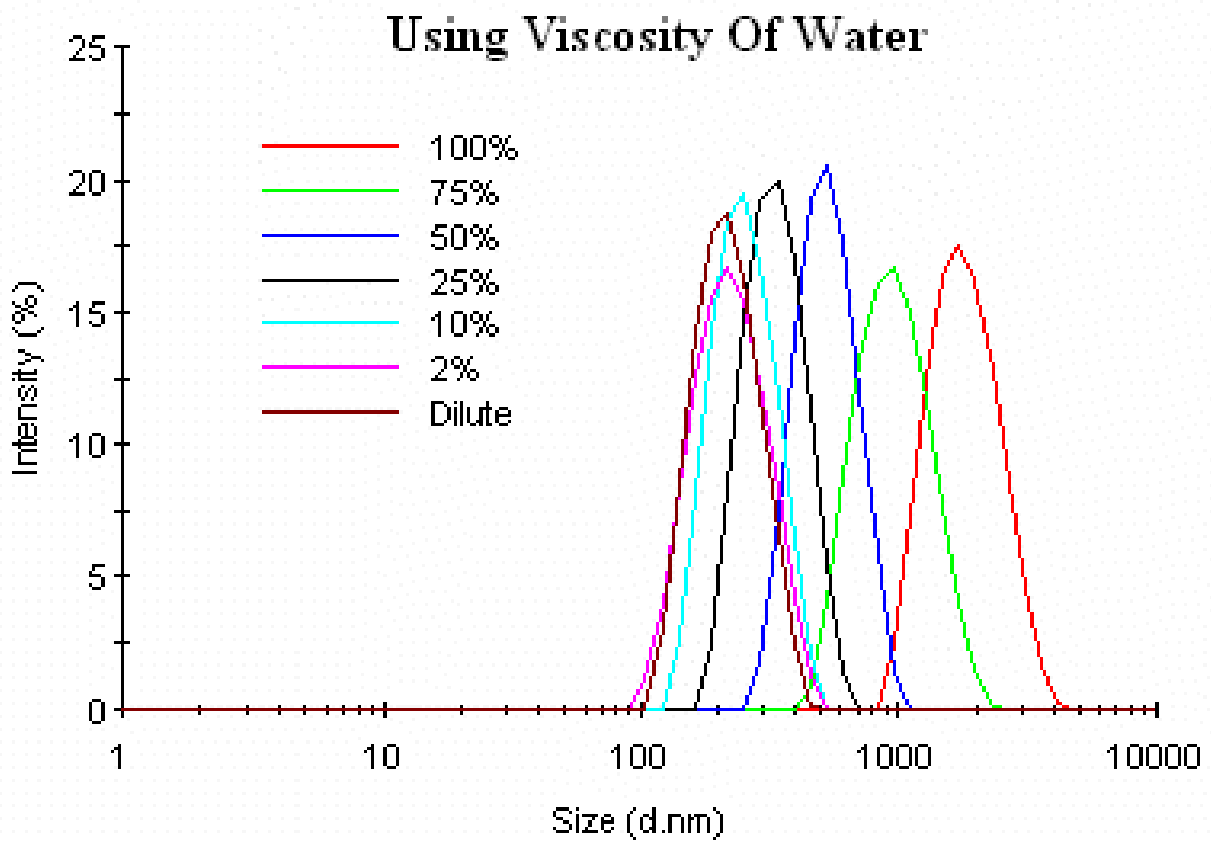
Suppress multiple scattering using specific systems in backscattering



Latex 200nm measured in backscattering and correct position in the cell
Even when the concentration increases the result remains unchanged.

If you don't use backscattering the size decreases quickly with mutiple scattering when concentration increases

Other example with restrictive diffusion



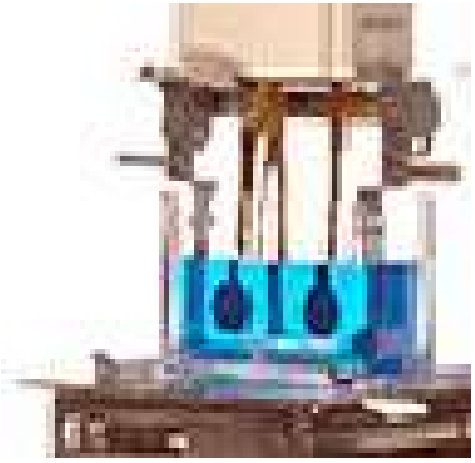
- With restrictive diffusion, the size of nanoparticles increases with concentration.

Well described in the ISO 22412 about DLS in concentrated samples

- Oil in alcohol emulsion (Baileys) if we use viscosity of water instead of bulk or sample viscosity the size of the sample increases with concentration !

Use correct Viscosity dynamic / kinematic

- The required viscosity for Henry equation is the dynamic viscosity (η) obtained by measuring kinematic viscosity (ν) multiplied by volumic mass (ρ) of the liquid:
- $\eta = \nu \rho$ (mPa.sec or centipoise)



For newtonian fluids
Direct measurement of η

Example of η for polyurethane suspension

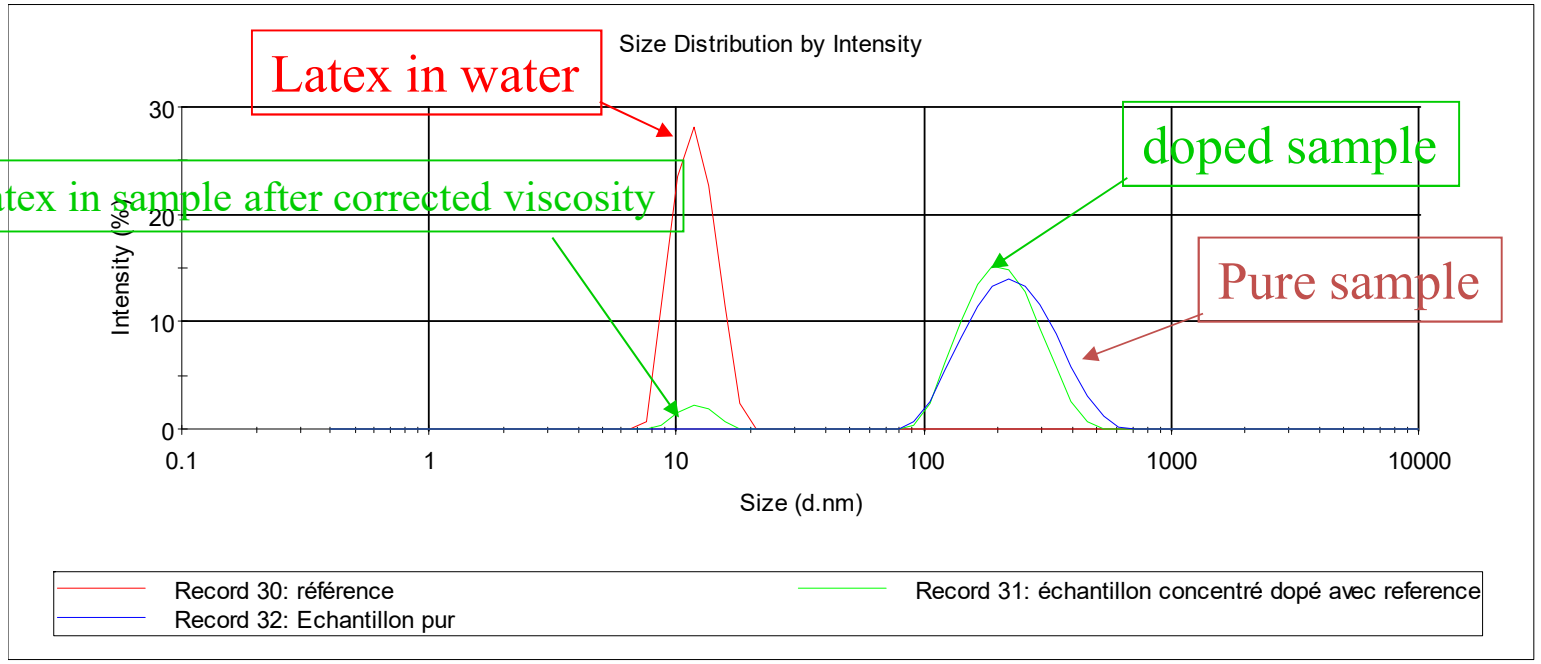
Concentration poids/vol	Viscosité mPa.s
pur 40%	**158**
33.3	14.5
26.7	5.52
20	2.77
13.3	1.74
10	1.49
6.7	1.17
5	1.1
3.3	1.02
1.7	0.89
0.7	0.89

How to measure η ?

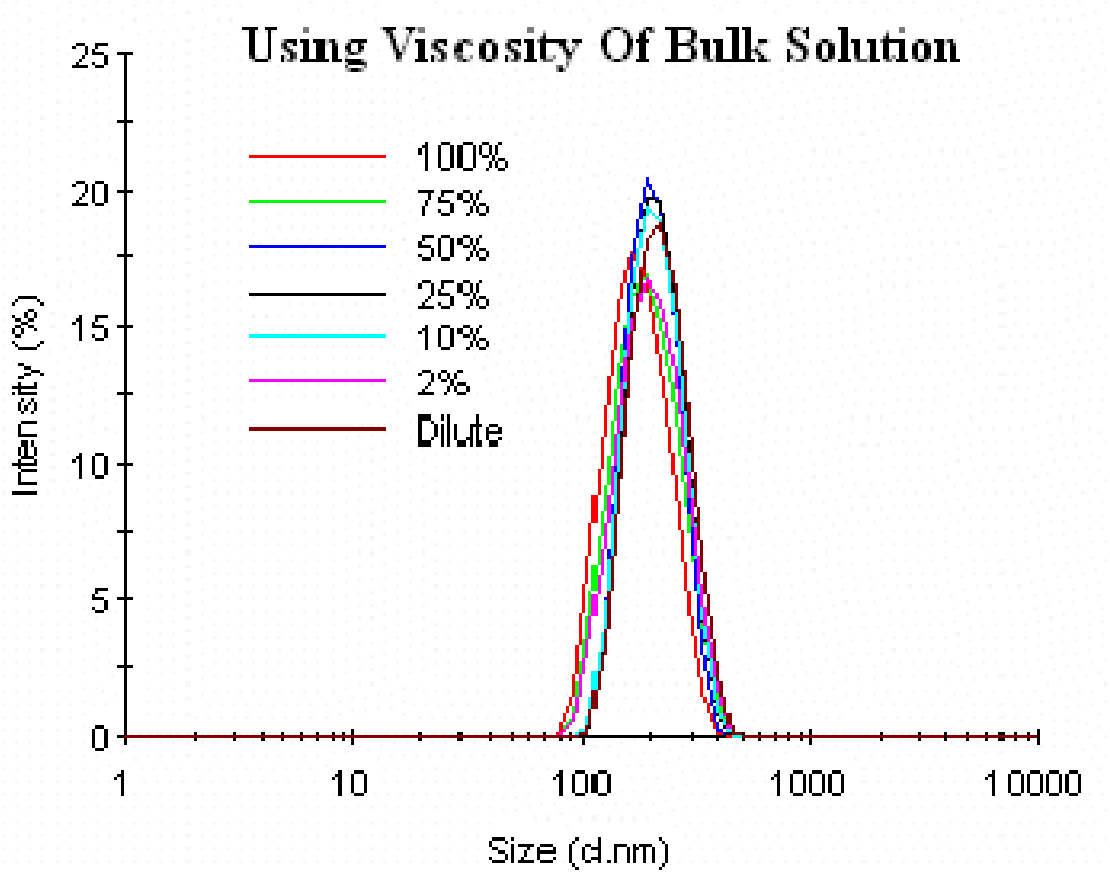
- Mix your sample with a latex of known size, (doping)
- Change the viscosity until the latex is at the right size.

$$d_{correct} = d_{mesuré} \times \frac{\text{viscosité initiale}}{\text{viscosité dynamique du milieu concentré}}$$

connu — $d_{correct}$ — connu — $d_{mesuré}$ — connu — viscosité initiale — inconnu — viscosité dynamique du milieu concentré

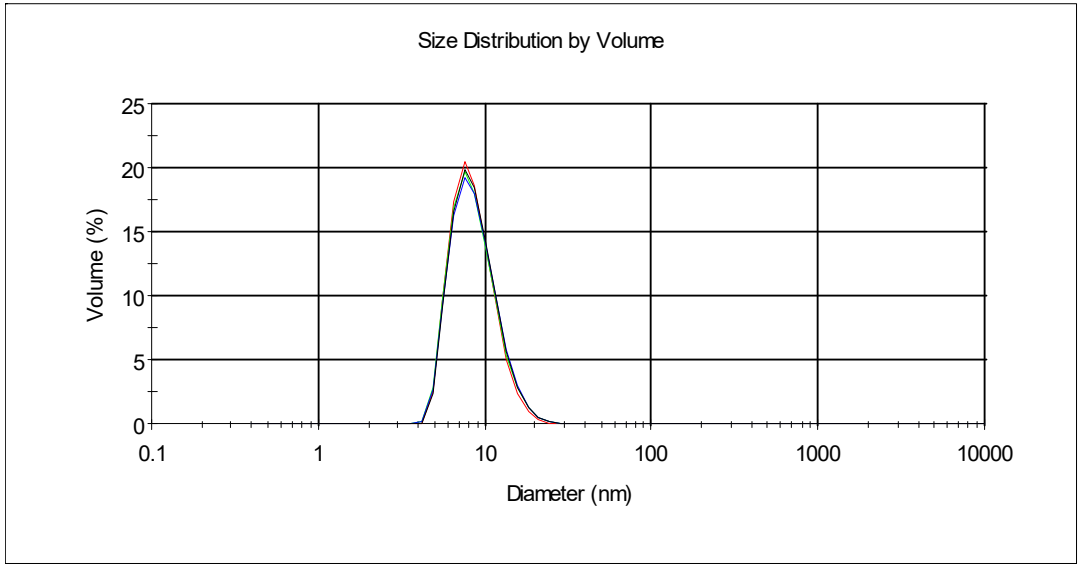


restrictive diffusion corrected by changing the viscosity



- If we use kinematic viscosity of the bulk sample, the size remains constant whatever is the concentration. It's an ideal case of restrictive diffusion with very few electrostatic effects. This is different compared to multiple scattering.

Precision: Measurement of a protein 50kDa



Conditions :

Concentration: : (0,3 mg/mL)

Refractive index : 1,33

Viscosity : 1,0563 cP

Temperature : 25°C

Attenuation of laser : none

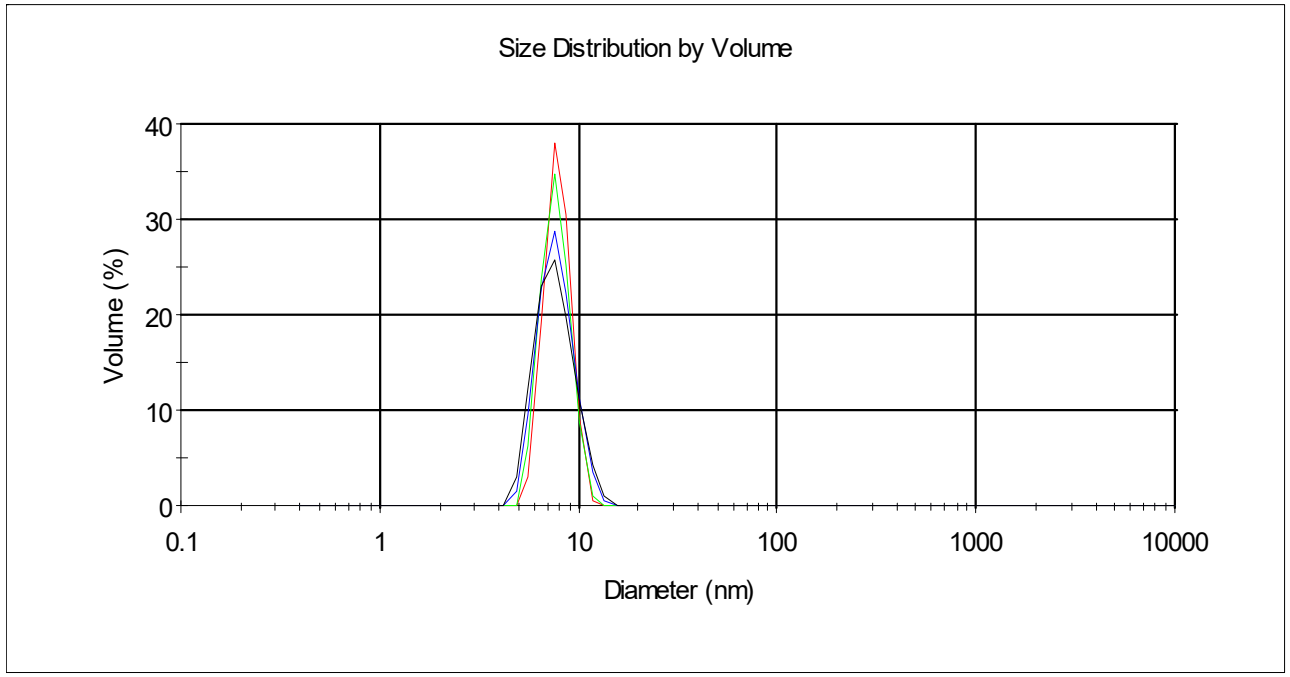
Position in the cell : 4,65 mm

5 independant measurements.

Measurement of exopolyphosphatase de T. Brucei under native form (50kDa) and truncated form without 150 amino-acids (35 kDa).

The native protein is measured at 8,88 nm by DLS

Precision: Measurement of truncated protein 35 kDa



Conditions :

Concentration: : (0,3 mg/mL)

Refractive index : 1,33

Viscosity : 1,0563 cP

Temperature : 25°C

Attenuation of laser : none

Position in the cell : 4,65 mm

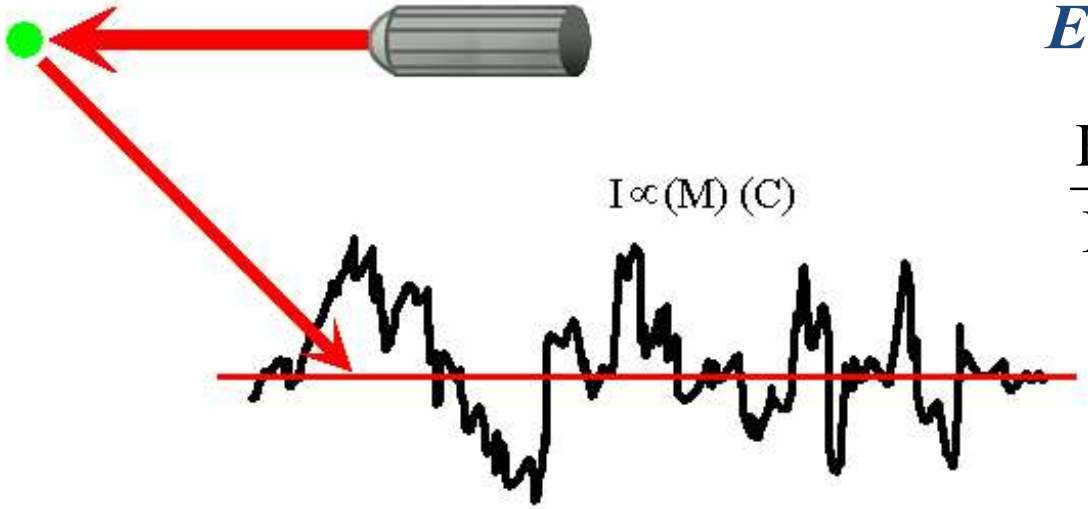
4 independant measurements.

The truncated protein is measured at 7,7 nm by DLS.

The precision can be better than 1 nm!

APPLICATION in SLS (static light scattering)

The mean scattered intensity allow calculation of molecular weight and 2nd virial coefficient.



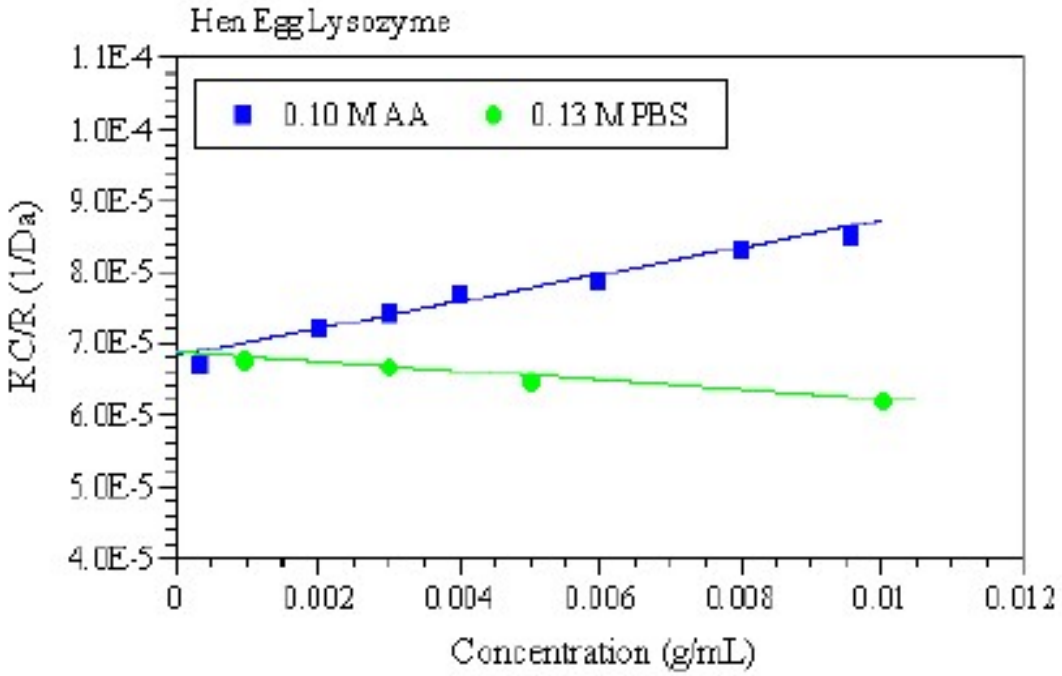
Equation de Rayleigh

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C\right)P(\theta)$$

K = Optical constant
M = Molecular weight
A_2 = 2nd virial coefficient

C = Concentration
R_q = Rayleigh ratio
P(q) = Structure factor

Measurement of molecular weight Mw & 2nd Virial Coefficient



For small molecules like proteins, the plot KC/R vs C concentration should be linear and intercept Y axis in a value which represents reverse of molecular weight (1/Mw).

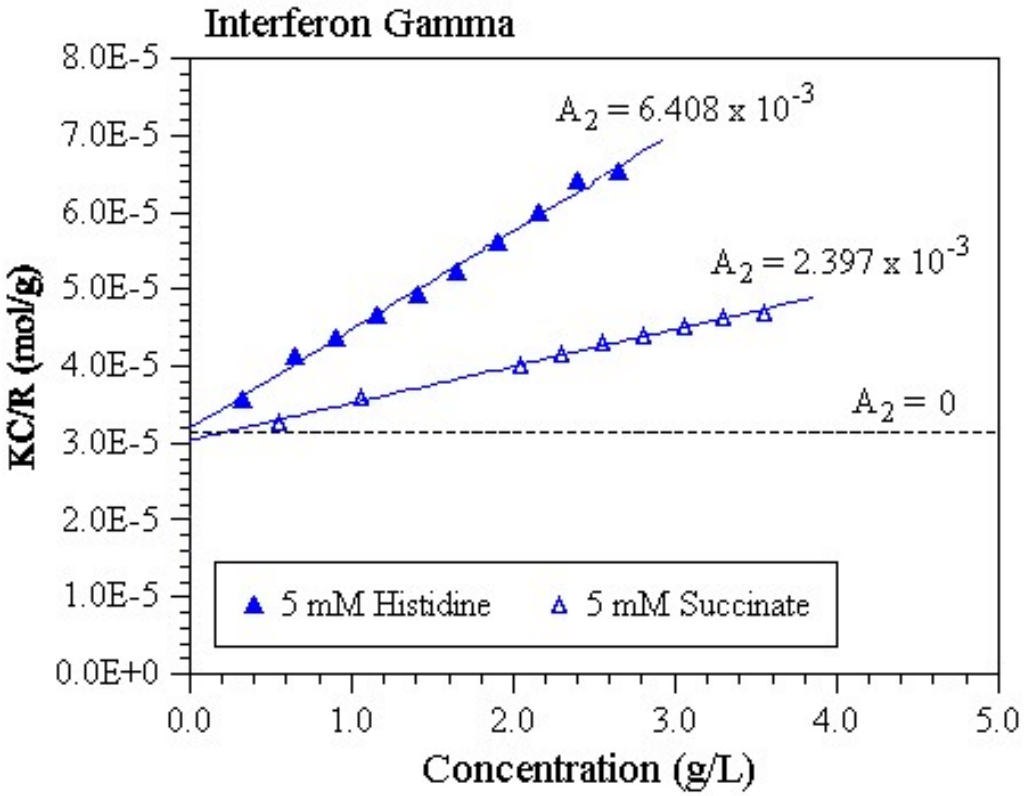
The slope is proportional to second virial coefficient.

This plot is like a Zimm plot at 1 angle only and is called a Debye plot.

With Debye plot , only the concentration changes.

2nd virial coefficient (A_2)

A_2 is determined from the slope of Debye plot.

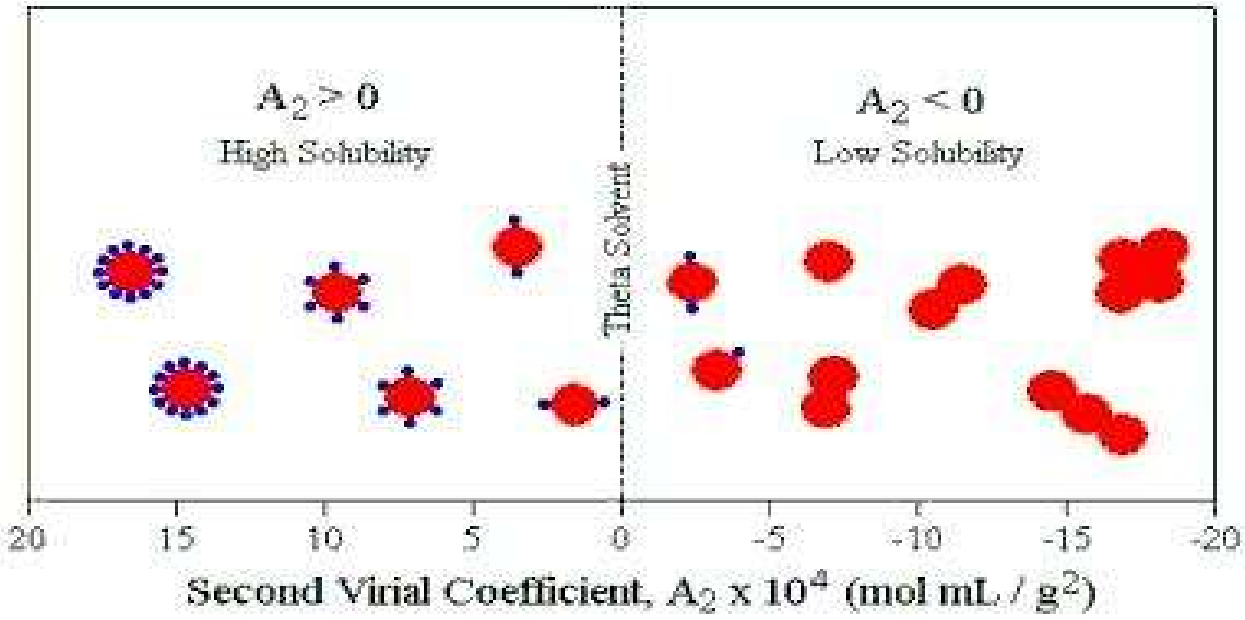


Equation for Debye plot

$$\frac{KC}{R_\theta} = \frac{1}{M} + 2A_2C$$

Explaining 2nd virial coefficient A_2

The second virial coefficient is representative of sample solubility



$A_2>0$: repulsive interactions between particles

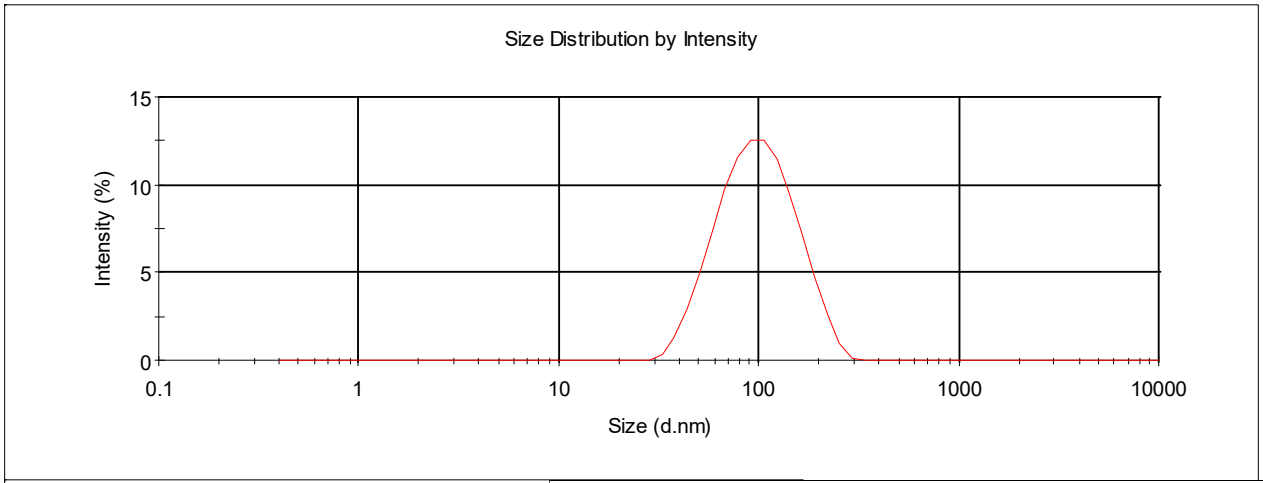
Good solvent and good stability of particles

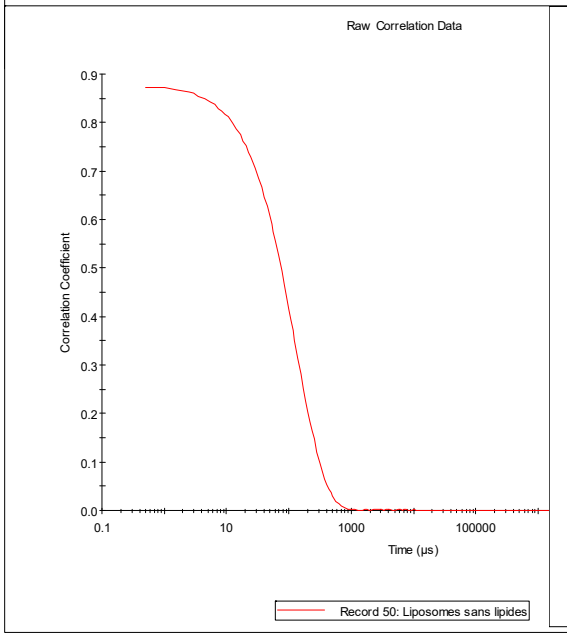
$A_2=0$: No interactions

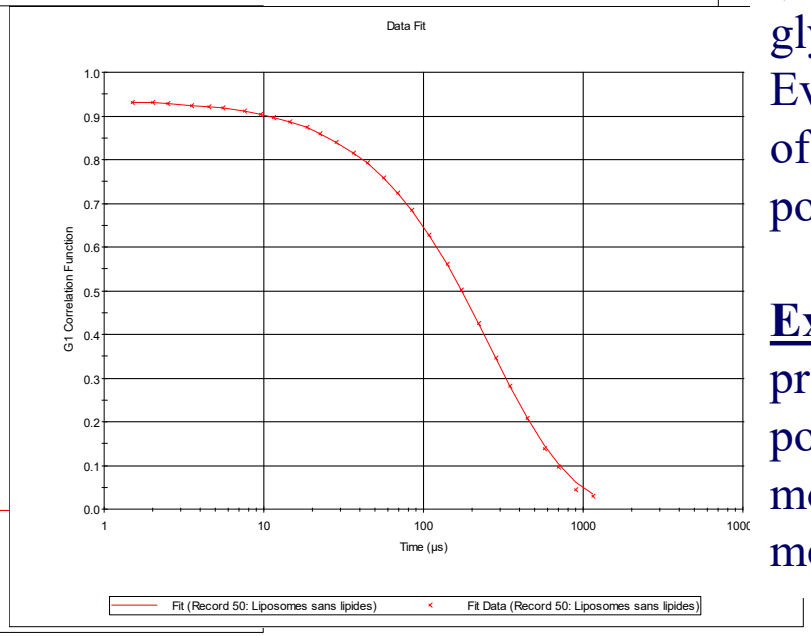
$A_2<0$: attractive interactions between particles

Bad stability of particles
Low zeta potential

Looking inside the nanoparticles!



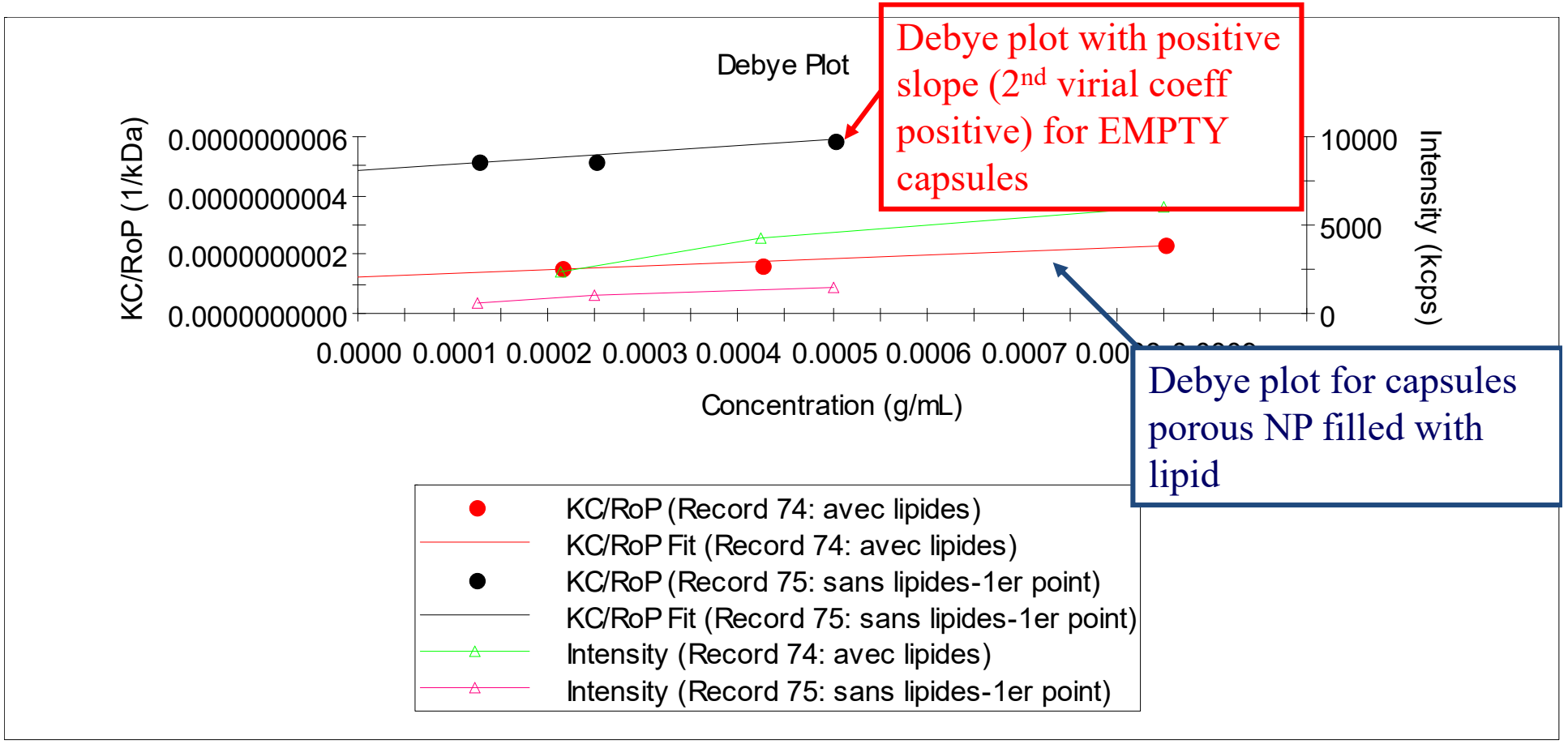




Nanoparticles polysacharides porous and cationic developped in Brain Blood Barrier Lab in Lens by Didier Betbeder and Youssef Jallouli.
NP are filled with anionic lipids DTPG (dipalmitoylephosphatidyl glycol).
Even with the negative charge of DTPG lipid, the zeta potential was not modified!

Expérience: Confirm presence of DTPG inside the pores of Nanoparticles by molecular weight measurements with SLS.

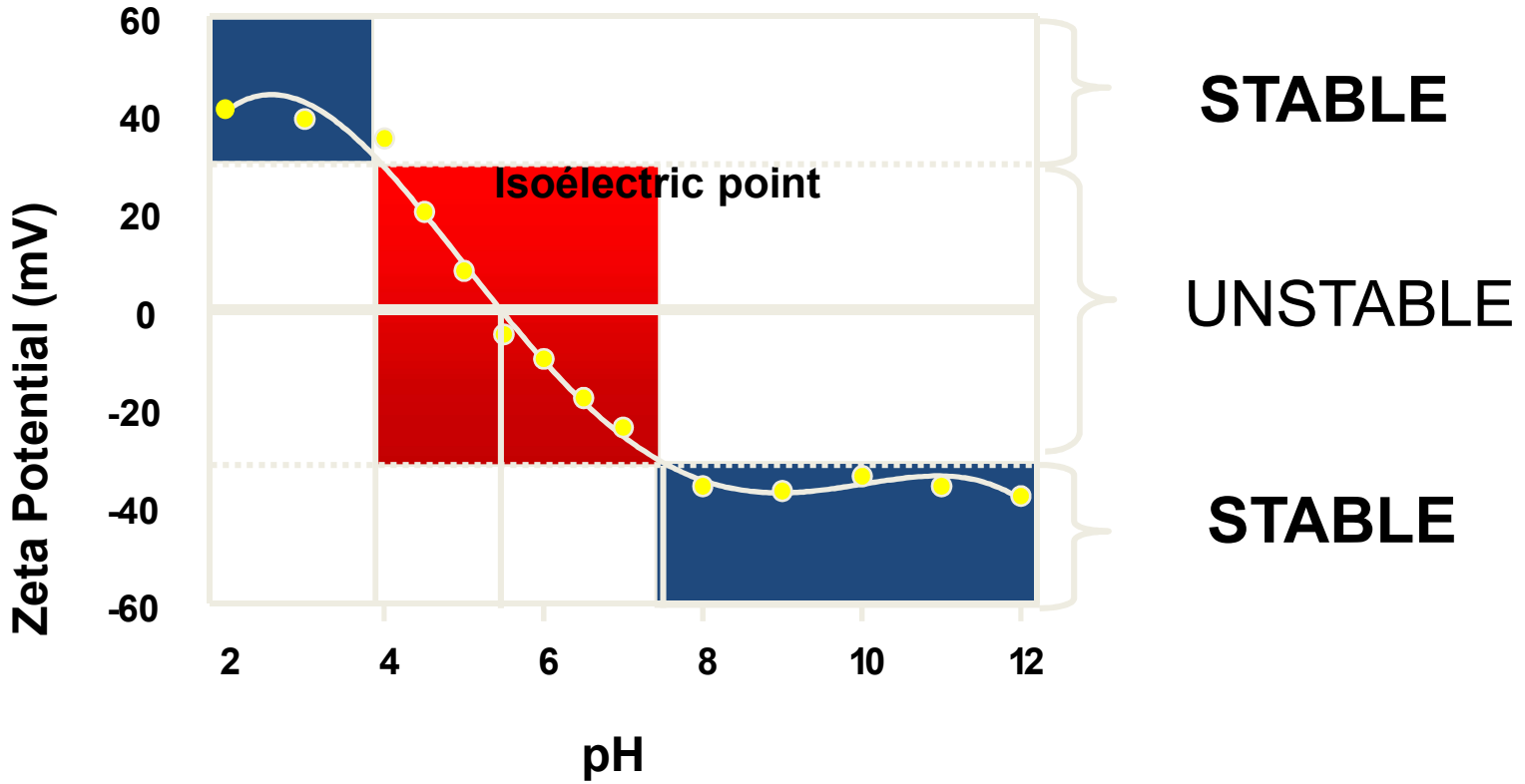
Using molecular weight



- Empty capsules Mw = 2000 kDa et A2 = 1 10^{-4}
- Filled capsules with lipid DTPG Mw=8000 kDa et A2 = 6,2 10^{-5} repulsive interactions and good stability.

Zeta Potentiel vs stability

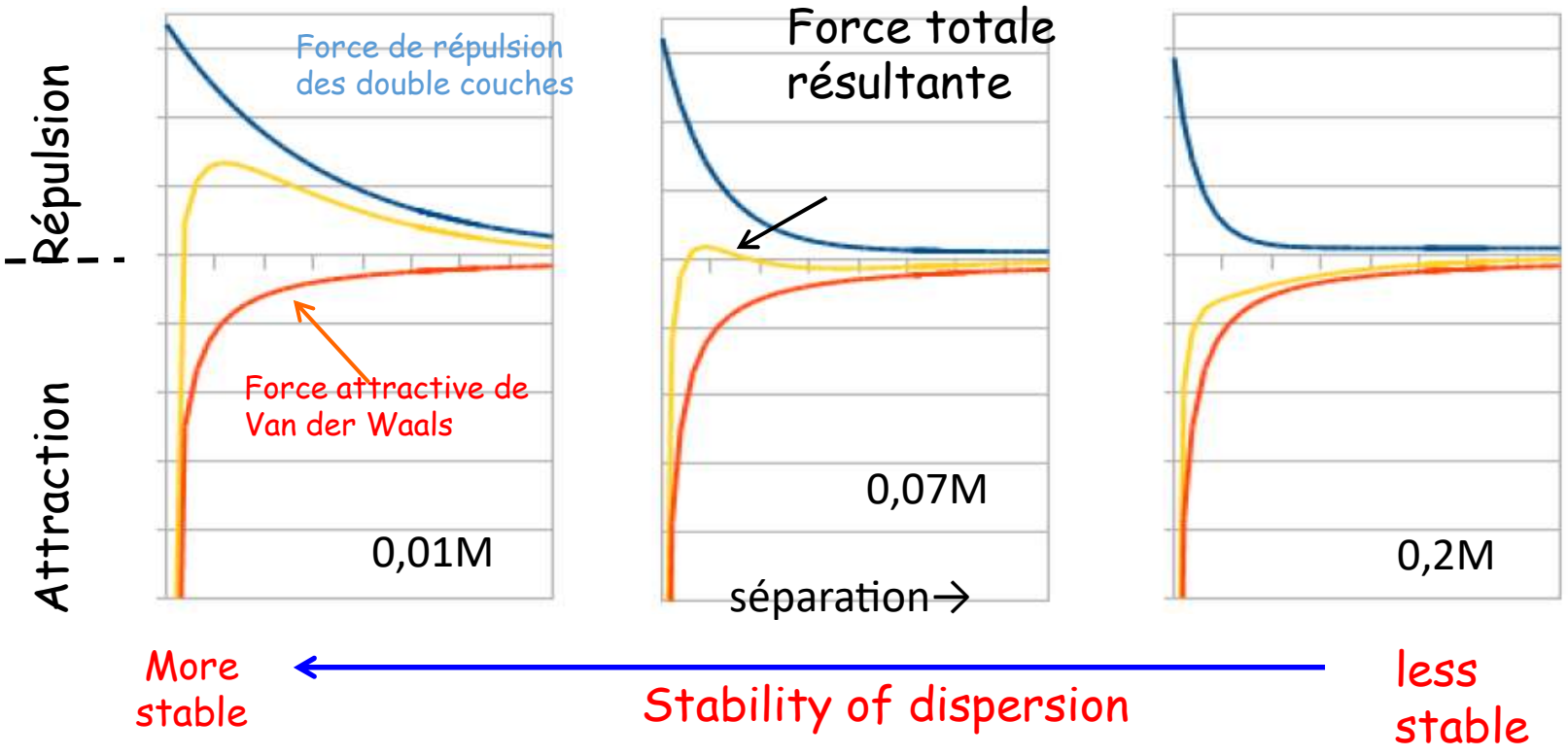
- Some people say that suspension is stable with zeta potential above 30 mV.
- This is not true!



DLVO Theory for Stability of suspensions

By Derjaguin, Verwey, Landau et Overbeek in 1940

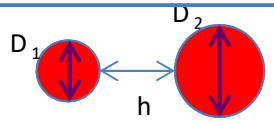
Total Force = attractive force from Van der Waals + répulsive force from double layers



Dispersion in air and water

Cohesive forces between particles are due essentialy Van der Waals forces

$$Attraction = A_{121}\frac{D_1 D_2}{6h(D_1 + D_2)}$$



A_{121}= constante d'Hamaker particules de matériau 1 plongées dans un milieu 2

	dans l'Air	dans l'Eau
Quartz	$A_{1\ air\ 1}$ = 6,50 x 10^{-20} joule	$A_{1\ eau\ 1}$ = 1,70 x 10^{-20} joule
Calcite	$A_{1\ air\ 1}$ = 10,1 x 10^{-20} joule	$A_{1\ eau\ 1}$ = 2,23 x 10^{-20} joule
PVC	$A_{1\ air\ 1}$ = 7,78 x 10^{-20} joule	$A_{1\ eau\ 1}$ = 1,30 x 10^{-20} joule
PTFE	$A_{1\ air\ 1}$ = 3,80 x 10^{-20} joule	$A_{1\ eau\ 1}$ = 0,33 x 10^{-20} joule

Hamaker constant : $A_{1\ air\ 1} \approx$ 5 times more than $A_{1\ eau\ 1}$

Attractive forces from Van der Waals between particles are 5 to 10 time less important in water compared to air.

This is more easy to disperse particles in water than in air

ISRAELACHVILI Jacob N., Intermolecular and surface forces, ACADEMIC PRESS, 1992

Hamaker Constant (few values)

In Water

$0{,}28.10^{-20}$ J for Pentane,

$0{,}49.10^{-20}$ J for Hexadecane

$0{,}65.\ 10^{-20}$ J for bacteria

$1{,}4\ .\ 10^{-20}$ J for Polystyrene

$1{,}7\ .\ 10^{-20}$ J for Silica

$2\ .\ 10^{-20}$ J for Mica

$4{,}2\ .\ 10^{-20}$ J for Alumina

$21\ .\ 10^{-20}$ J for Iron Oxide

$26\ .\ 10^{-20}$ J for TiO2 (rutile)

$40\ .\ 10^{-20}$ J for Gold

Gold, Copper, Silver 30.10^{-20} et 40.10^{-20}

- In Air

$6\ .\ 10^{-20}$ for Polystyrene,

10.10^{-20}J for Mica

14.10^{-20} for Alumina

21.10^{-20} for Iron Oxide

43.10^{-20} for TiO2 (rutile)

44.10^{-20} for Silicon Carbide

Gold, Copper, Silver

25.10^{-20} to 40.10^{-20} J (in theory)

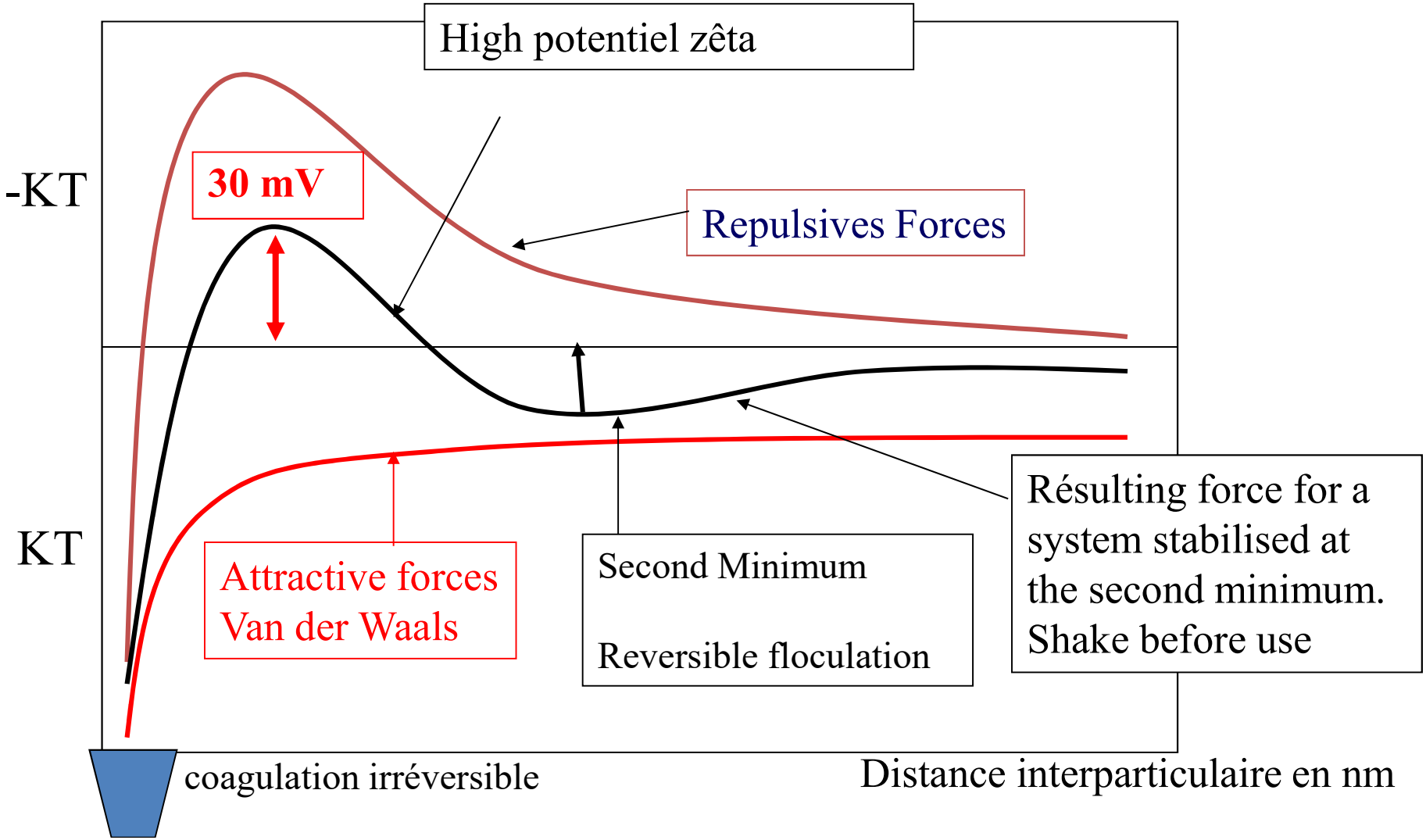
30.10^{-20} to 50.10^{-20} (experiments)

Solvents between 3.10^{-20} to $5{,}5.10^{-20}$J

water $3{,}7.10^{-20}$ J

System stabilised at the second minimum

Total energy of interaction is the combination of attractive force and repulsive forces described by DLVO.



Conclusion

- With DLS you can measure distribution of nanoparticles size weighted by scattered intensity
- With SLS you can measure Mw up to 2.10^7 Da (30 nm)
- With 2^{nd} virial coeff from SLS you measure solubility and stability behaviour
- With Debye plot from SLS you can « see » inside the particle if it is full or empty
- With zeta potential you measure affinity with stabilizers and stability in suspension