

Short Introduction to Static Light Scattering

Static Light Scattering using the ALV/CGS-3 Compact Goniometer System

ALV-GmbH Laser Vertriebsgesellschaft mbH

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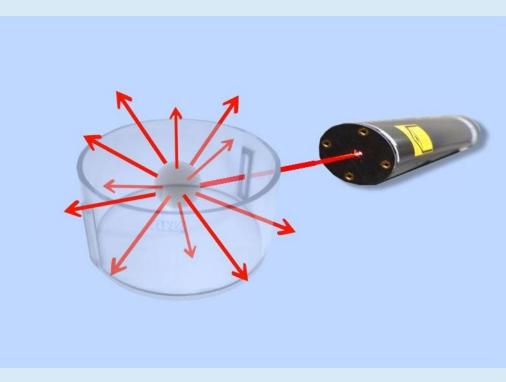
1.0. Static Light Scattering (SLS)

- a classical method for the determination of *Molecular Mass*, shape and (possible) interaction (*Radius of Gyration*, *Second Virialcoefficient*) as well as superstructure (*Static Structure Factor*) of particles in solution
- is used (among others) for the determination of the *Aggregation Number* (number of molecules present in a structure) of micelles or polymer coils
- requires an *angular dependent* measurement of the *scattered light*
- SLS measurements are (in conclusion) limited to the analysis of *monomodal systems*, as the dependency of the scattering intensity increases with the sixth power of the particle diameter, so larger contributions (like e.g. aggregates) would highly interfere the mean intensity result from the particles
- therefore very clean and almost *dust free measurement conditions* are required



1.1. Brief theory of Light Scattering

Light Scattering is caused by the *interaction of electrons of an isotropic particle/molecule* with an influencing alternating electromagnetic field (light wave), which sets the electrons in oscillation. Hereby, dipoles with orientation towards the polarization plane of the incident light are induced (Hertz dipoles), which consequently emit a corresponding light field of the same frequency, that shows on time average a characteristic radiation pattern.

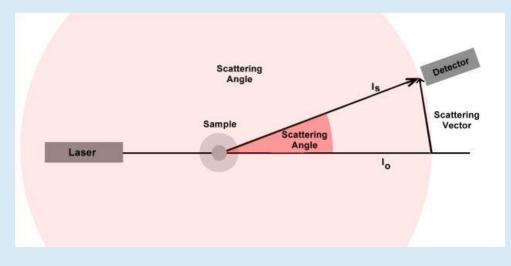




The distance and angular dependency of such a Hertz dipole is given as:

$$\frac{I_s(r,\theta)}{I_0} = \frac{16\pi^4 \alpha^2 \sin^2 \psi}{\lambda_0^4 r^2}$$

- $I_{\rm S}$ = Scattering intensity
- I_0 = Intensity of the incident light
- Θ = Scattering angle
- α = Polarizability
- ψ = Angle between dipole axis and direction of observer (detector)
- r = distance to observer (detector)
- λ = wavelength of the incident light



The scattering vector *q* derives from:

$$\left|q\right| = \frac{4\pi n_d \sin(\theta/2)}{\lambda}$$





The **fluctuation theory of Einstein** solves the contradiction that in a homogeneous system, scattered light can be detected in a homogeneous system despite the fact that in a large scattered volume there always exist corresponding partial volumes which produce a 180 ° phase-shifted scattering and thus destroy all scattered light destructively. The reason for this is the spatial and temporal fluctuation of the polarizability. This fluctuation in a partial volume dV can be attributed to a fluctuation of the concentration.

As an important factor here the refractive index increment

i.e. the change in the refractive index (dn) with the concentration (dC) at a constant temperature, is taken into account.

For linear polarized light and a particle size of $\leq \lambda/20$ this results in

$$I_s = I_0 \frac{4\pi^2 \left(\frac{dn}{dC}\right)^2 M^2}{\lambda^4 r^2 A^2}$$

A = Avogadro constant M = Molecular weight of the particles

taking into account the refractive index of the solvent n_0 (for correcting the vacuum-related wavelength of the incident light) and the particle number *N* per volume

$$N = \frac{CA}{M}$$

A = Avogadro constant

dn

dC

M = Molecular weight of the particles

C = Particle concentration



One obtains for the scattering intensity per (detection) volume unit

$$I_s = I_0 \frac{4\pi^2 n_0^2 \left(\frac{dn}{dC}\right)^2 MC}{A\lambda^4 r^2}$$

with the so called **Rayleigh Ratio** $R_{\mathfrak{F}}$

$$R_{g} = \frac{I_{s}}{I_{0}} \cdot \frac{r^{2}}{2}$$

In which the scattering intensity must take account of the fact that the detector, as a function of the scattering angle θ , in each case considers a different scatter volume (for this purpose, the measured scatter intensity $I_{Messung}$ according to

$$I_{s} = I_{Messung} \cdot \sin \vartheta$$

Correspondingly converted to the surface-normalized scattering intensity), and the introduction of an optical constant K

$$K = \frac{2\pi^2 n_0^2 \left(\frac{dn}{dC}\right)^2}{A\lambda^4}$$



the fundamental equation of light scattering is obtained

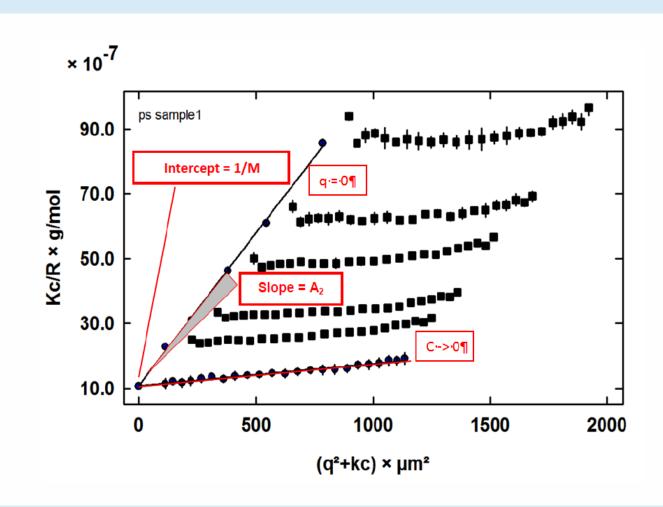
$$\frac{KC}{R_{g}} = \frac{1}{M} + 2A_{2}c +$$

 A_2 = second virial coefficient

It applies that A_2 is negative for attractive interaction and A_2 is positive for repulsive interactions

When applying *Kc* / *R* for different concentrations against the mass concentration *c* and subsequent extrapolation to small concentrations, the axis section (intercept) yields the inverse molar mass 1/M. From the slope of the extrapolation line q-> 0, derived from the extrapolations of the concentrations to q-> 0 the value for the second virial coefficient A_2 is obtained





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Static Light Scattering using the ALV/CGS-3 Compact Goniometer System

For particles larger than $\lambda / 20$ intramolecular interferences occur due to the presence of several scattering centers, i.e. light from different partial regions of the particle is in a fixed phase relation to one another. However, the interference is dependent on the measured scattering angle, and for two scattering centers it can be shown that the phase shift *j* is related with the scatter vector *q*

$$\left|q\right| = \frac{4\pi n_d \sin(\theta/2)}{\lambda}$$

and the distance r of the two centers

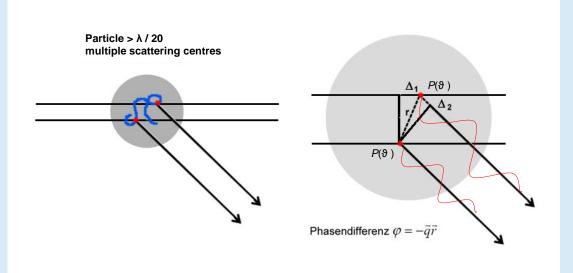
$$\varphi = -\vec{q}\vec{r}$$

As a result, more light is scattered into forward direction (0 °) than into back direction (180 °). Thus, e.g. the measured scattering intensity of a larger particle (> λ / 20) at *q* = 45 ° is higher than at *q* = 135 °, although the Rayleigh theory predicts the same intensity.

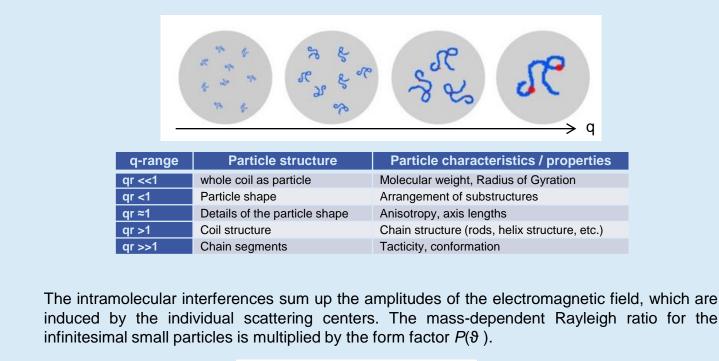




The intramolecular interferences depend essentially on the phase shift so that the interference is influenced by the change in the scattering angle and thus *q*. Also, by changing the wavelength λ of the incident light, *q* can be varied. For larger wavelengths, *q* becomes smaller, i.e. in order to make smaller sub-regions of the to be measured particle (e.g., polymer or nanoparticles) visible, and as a result the distances *r* of the interfering scattering centers seem to be reduced, the scattering vector *q* must be increased with a constant phase shift.







$$R_{g}(realesTeilchen) = P(\mathcal{G}) \cdot R_{g}$$

In general, P (θ) is averaged over all possible spatial orientations of the particles (by the Brownian molecular motion) as well as all possible distances of the scattering centers

$$P(\mathcal{G}) = \frac{1}{N} \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{\sin(qr_{ij})}{qr_{ij}}$$

N = Total number of scattering centers

> q

r = Distance of the scattering centers





The fundamental equation of light scattering is corrected by the above mentioned **Debye** interference theory to

$$\frac{Kc}{R_{\theta}} = \frac{1}{MP(\theta)}$$

The scattering actuator P(q) varies with the radius of gyration R_q according to

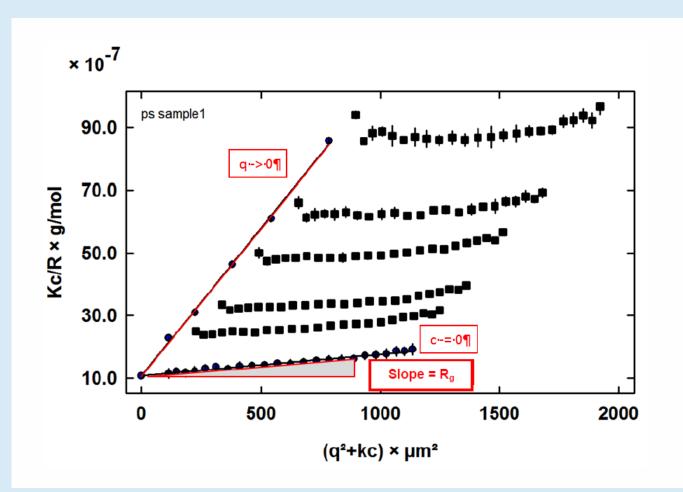
$$P(\theta) = 1 - (4\pi/\lambda_0 \sin\theta/2)^2 \frac{\langle R^2 \rangle}{3} + \dots$$

This results in the fundamental equation to

$$\frac{Kc}{R_{\theta}} = \frac{1}{M} \left(1 + (4\pi/\lambda_0 \sin\theta/2)^2 \frac{\langle R^2 \rangle}{3} \right)$$

From the plot of Kc/R versus q respectively q^2 with extrapolation to q = 0, the gyration radius R_g is obtained from the slope of the extrapolation straight line, derived from the extrapolations of the concentrations to $c \rightarrow 0$









Thus, the characteristic course of the angular dependent scattering intensity provides information about the shape and size of the scattering particle. Examples of form factor models are (among others):

Monodisperse Coil Polydisperse Coil

Thin Rod

Hard Sphere

$$P_{Z}\left(u=qR_{g}\right) = \left(\frac{2}{u^{4}}\left(\exp\left(-u^{2}\right)-1+u^{2}\right)\right)^{2}$$

$$P_{Z}\left(u=qR_{g}\right) = \left(1+\frac{u^{2}}{3}\right)^{-1}$$

$$P_{Z}\left(u=qL=12qR_{g}\right) = \frac{2}{u}\int_{0}^{u}\frac{\sin\left(u\right)}{u}du - \left(\frac{2}{u}\sin\left(\frac{u}{2}\right)\right)^{2}$$
L length of the rod,
$$P_{Z}\left(u=qR_{g}=\sqrt{\frac{5}{3}}qR_{g}\right) = \left(\frac{3}{u^{3}}\left(\sin\left(u\right)-u\cos\left(u\right)\right)\right)^{2}$$
Rs radius of the sphere,



2.0. Performing a Static Light Scattering measurement

Sample Preparation

- preferably monodisperse particles
- determine an appropriate solvent (organic / aqueous, etc.)
- an accurate sample weight and volume determination for exact sample concentration
- determine/measure the refractive index of the solvent
- determine/measure the refractive index increment (dn/dc) of the particle in the corresponding solvent -> measurement with a differential refractometer
- perfect solution (of particles) required -> no loss of concentration
- filtration of the sample (PTFE filter for organic solvents / CA filter for aqueous solvents), use filter with appropriate pore size
- Preparation of concentration series required (at least for determination of molecular weight)



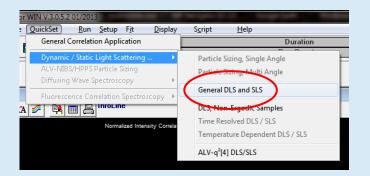
2.1. Performing a SLS measurement using the ALV-Correlator Software



1.) Select the angular dependent measurement mode via Quick Set

📑 AL	V-7004	Correla	tor So	ftware f	or WIN V.3.0.5.2	01/201	.3		_					-
File	Edit	Windov	V	Samp	e QuickSet	Run	Setup	Fit	Dis	olay	Script	Help		
			ä										Duration	
					2								Run Counter	
\$	/%к		Panel	ø						Ð		8		

2.) Click on Dynamic/Static Light Scattering and subsequently select General DLS and SLS





3.) Angular Dependent DLS&SLS measurement mode

Min Anala, May Anala, Anaulas Chan		Lower	Upper	Step	Runs
Min. Angle Max. Angle Angular Step 30.00 150.00 10.00	Range 1	201101	oppor	Citop	- Auto
	Range 2				
Runs Duration[s] 3 á 10 Accept this Set ➡	Range 3				
Remeasure if dRate exceeds 5 %	Range 4				
Sample Setup Ecumulant Atten.	Range 5				
STEP 2 Static & Dynamic LS Options	STEP	3 Mea	surement	Туре	
Measure and correct for detector dark counts	•	Standard	C Solver	nt 🔿 Sa	olution
Use Toluene Rayleigh Ratios as standard	Standa	rd File : 🛛	STANDARD	TOL	
presuming a Refractive Index of 1.4954		F			<u> </u>
Compute Rayleigh Ratio for W-Scattering	Solven	tFile: 9	SOLVENT.T	OL	<u>👄</u>
Instead use this RR : [1,0e-5 [1/cm]	Solutio	n File : 🛛			
Correct for index matching fluids refrative index	I 🗆 Me	asure without	t solvent info	ormation/fi	
Refrative Index = 1.4954		pend solution			0
	Cre	ate Angular D) ependent F	Report	
Compute and store Cumulants Analysis	Sample	Concentratio	on: 1ϵ	ə-3	[a/ml]
Data cutoff at : 10 % of maximum					
Generate measurement protocol	i Sample	edn/dc :	0.	1	[ml/g]



4.) Enter the favored measurement sequence in the Measurement Control section

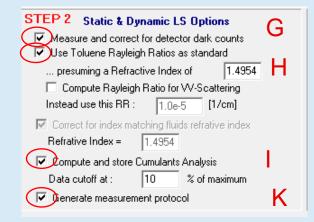
ngular Dependent Dynamic and Static Light Scattering							
Angular Dependent Dynam	ic and St	tatic Lig	ht Scatte	ring		_	
STEP 1 Measurement Control							
Min Angle Max Angle Angular Step		Lower	Upper	Step	Runs		
30.00 150.00 10.00 A	Range 1						
Buns B Duration[s]	Range 2						
3 á 10 Accept this Set ➡	Range 3						
remeasure if dRate exceeds D 5 %	Range 4						
Sample Setup ECumulant Atten.	Range 5						

- A) enter the starting angle (*Min. Angle*) and the final angle (*Max. Angle*) as well as the angular steps (in °)
- B) enter the number of runs (*Runs*) at each measurement angle, as well as the duration of each run (*Duration*)
- C) transfer the settings with *Accept this Set* to the measurement sequence chart
- D) if required use a "dust-filter" function (standard deviation of the mean intensities) via *Remeasure if dRate exceeds … %*
- E) enter the sample parameters (refractive index and viscosity of the solvent) or select an existing solvent from the schedule of the **Sample** menu
- F) select the correlator settings (Auto/Cross Correlation, etc.) in the **Setup** menu

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5.) Setting the measurement parameters in section Static & Dynamic LS Options



- G) tick the *Measure and correct for detector dark counts* box for the prior measurement of the detector's dark counts
- H) select the standard value for the *Rayleigh Ratios* of toluene
- activate the Compute and store Cumulants-Analysis function in order to determine the hydrodynamic radius R_H derived from the Cumulahnt Fit of the simultaneous DLS measurement
- K) select **Generate measurement protocol** to get the measurement data/graph indicated in the *Ratio/Scattering Angle Display*



6.) Selecting the measurement mode using the *Measurement Type* menu

STEP 3 Me	asurement Type						
• Standard C Solvent C Solution							
Standard File :	STANDARD.TOL						
Solvent File :	SOLVENT.TOL 😁						
Solution File :	<u> </u>						
🔲 Append solutio	 Measure without solvent information/file Append solution data to solution file Create Angular Dependent Report 						
Sample Concentra	tion : 1e-3 [g/ml]						
Sample dn/dc :	0.1 [ml/g]						

L) Select the **Standard** mode for the standard measurement of toluene and safe the data as Standard.TOL file (or use a different file name) respectively use a file of an already existing toluene measurement as Standard File



Click on Start Measurement to start the measurement !



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	asurement Type	/					
○ Standard ⓒ Solvent ○ Solution							
Standard File :	STANDARD.TOL 😕						
Solvent File :	SOLVENT.TOL	\mathbb{D}					
Solution File :	<u> </u>	•					
 Measure without solvent information/file Append solution data to solution file Create Angular Dependent Report 							
Sample Concentral	tion : 1e-3 [g/ml]						
Sample dn/dc:	0.1 [ml/g]						

M) Select the **Solvent** mode for the measurement of the (pure) solvent used for the preparation of the sample solution and safe the data as *Solvent*.TOL file respectively use a file of an already existing solvent measurement as Solvent File



Click on Start Measurement for starting the measurement !

<u>Please note:</u> The prior measurement of the (pure) solvent serves to determine the scattering intensity contribution of the solvent, in order to subtract it lateron from the total intensity to get the excess scattering of the sample. The measurement of the solvent's intensity is only usefull in those cases, where the scattering intensity of the solvent has already an significant amount of the total intensity. In any other cases this step can be skipped.





STEP 3 Measurement Type C Standard C Solvent () Solution						
Standard File :	STANDARD.TOL					
Solvent File :	SOLVENT.TOL					
Solution File :	Test.STA					
Append solution data to solution file Create Angular Dependent Report						
Sample Concentra	tion : 1e-3 [g	g/ml]				
Sample dn/dc :	0.1 (r	ml/g]				

- N) for the measurement of the sample solution select the measurement mode **Solution** and safe the corresponding data as *.STA data file (Solution file)
- O) In case no Solvent File was priorly measured (or loaded) tick the Measure without solvent information/file box the scattering intensity of the solvent will be assumed as zero.
- P) Select *Append solution data to solution file* in case several sample concentrations will be measured and should be finally safed in one measurement file
- Q) **Create Angular Dependent Report** creates a report of angular dependency including distribution functions



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STEP 3 Me	asurement Type				
O Standard	🔿 Solvent 💿 Solution	ı			
Standard File :	STANDARD.TOL	0			
Solvent File :	SOLVENT.TOL	0			
Solution File :	Test.STA	<u> 👄</u>			
 Measure without solvent information/file Append solution data to solution file Create Angular Dependent Report 					
Sample Concentra	tion : 1e-3 [g/m				
Sample dn/dc :	0.1 [ml/				

- R) enter the value of the sample concentration (g/ml) in the **Sample Concentration** section
- S) enter the value of the refractive index increment (ml/g) of the sample particles (for the corresponding solvent used) in the **Sample dn/dc** section



Click on Start Measurement to start the measurement !



Statische Lichtstreuung mit dem ALV/CGS-3 Kompakt Goniometer System

Values of the refractive index increment in corresponding solvents



for typical sample/particle classes dissolved

Sample/Particle class	Solvent	dn/dc [mL/g]	
Lysozyme	Water /water based buffer	0,185	
Phospholipide (Liposome)	Water	0,16	
Polystyrene	cis-Decaline	0,12	
Polystyrene	Toluene	0,08 – 0.11	
Polystyrene	Tetrahydrofurane (THF)	0,18 - 0,19	
Polymethylemethacrylate (PMMA)	Tetrahydrofurane (THF)	0,09	
Polymethylemethacrylate (PMMA)	Toluene	0.01-0.02	
Polyvinylchloride (PVC)	Tetrahydrofurane (THF)	0,10	
Polyethyleneglycol	Water	0,13	



3.0. Performing a SLS measurement using the ALV/Fit & Plot Software



Open the *.STA file of the measured sample via *File -> Load New File*

ALV / Static & Dynamic FIT a File dit Window Help	nd PLOT		
Image: NoName k: _≤< ≥>			
Mw(c): Mw(q ²): Rg:	Loading File ! Please Wait ! Abort Loading	Gffnen Suchen in: ALVStat	 • ← 1 →
A2:		Name Ps_std1.sta Ps_std2.sta	Änderungsdatum 31.10.2008 19:50 24.03.1998 13:02
		Dateiname: ALV5000 [statdyn.] (*.sta;*.dat)	Offnen Abbrechen





LV-Stat: ALV-5000 Data		
Fixed Parameter No. of Data Record 2	2	Continue without any change Abort
Name of standard sample "Samplename needed."		<u> </u>
Name of solvent sample "Samplename needed."		(1) Prompt again after reading the next concentration
Temperature/K 29	295.904	(2) Perform for all records/concentratic do NOT prompt again. This is relevent arbuin construction under the second se
Adjustable Parameters		only in case of concentration value itself not has been edited here.
n-Standard 0.	.00001	Otherwise program will turn to option Change
n-Solvent 1.	.49600	Attention ! Make a copy of the
<u>d</u> n/dc / 1/(g/dm ³)	.06000E-04	original data file before pressing "Change", because recalculated
Concentration / g/dm ³ 6.	6.70000	data immediately overwrite the
Wavelength / nm 53	532.00	original data in the file !
Rayleigh Ratio RR / 1/m 2.	2.01700E-03	
Viscosity / mPas (cP	.66330E-01	
Sample name		
ps sample1		
dm³ = litre		





The *File Parameter Menu* shows a summary of the fixed respectively modifiable measurement parameters of the sample measurement – fixed (unchangeable) parameters are:

- selected Standard.TOL file
- selected Solvent.TOL file

Modifiable parameters (in retrospec) are:

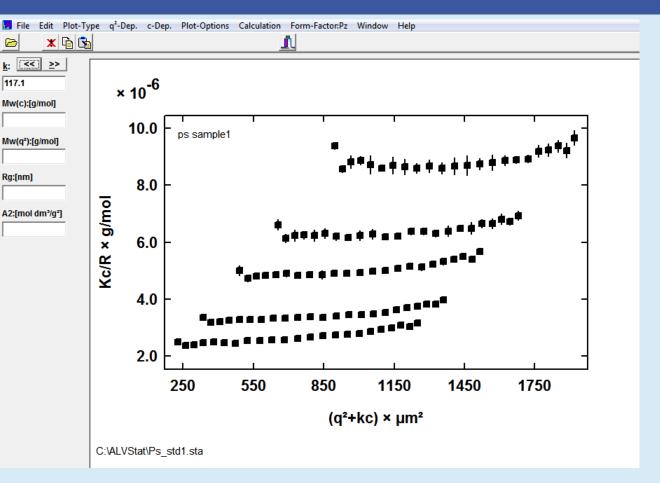
- refractive index Standard (typically toluene)
- refractive index solvent (for DLS)
- refractive index increment dn/dc of sample particles for corresponding solvent
- sample concentration
- wavelength of used light source
- Rayleigh Ratio of the standard (typically toluene)
- viscosity of solvent (for DLS)
- sample name

The sample measurement file can be opened either without any change or previous used parameters will be changed (1) individually for each concentration measurement or (2) for all subsequent concentration measurements (of the same sample)





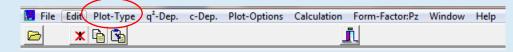




The file data is indicated in the preset Zimm-Plot (Kc/R versus q^2+kc), by using the spread factor *k* the q-functions of each measured concentrations can be streched in order to get the functions more properly separated.



The appropriate Plot-Typen can be selected via the *Plot-Type* function; there are the following Plot-types for the evaluation of the Static Light Scattering measurements available:



Zimm-Plot (Kc/R vs. q²+kc)

- Suitable for mono-disperse particles with particle size in the range of 20-50nm

The Zimm-Plot is a graphical evaluation method for scattering functions under consideration of the polydispersity of the macromolecules. Hereby the weight averaged molecular weight M_W can be derived from the scattering function *Kc/R* by double extrapolation, using the extrapolations $c \rightarrow 0$ (condition without any particle interactions) and $\theta \rightarrow 0$ (condition without scattering interference) System). To get these functions the intensity *I* of the scattered light for different sample concentrations (same sample material) will be measured at different scattering angle positions (respectivelly q-vectors) as an angular dependent measurement. The measured intensities are referred to the priorly measured *Standard* (typically toluene) and actually expressed as Reduced Scattering Intensity I_{red} .

The obtained intensity values will be converted into ratio values and subsequently transfered in a plot, whose ordinate (K·c/R) and abscissa (q² + k ·c) are depicted. After that the data points, which were measured at the same concentration respectively at the same scattering angle, will be interconnected.





The file data is indicated in the preset Zimm-Plot (Kc/R versus q^2+kc), by using the spread factor *k* the q-functions of each measured concentrations can be streched in order to get the functions more properly separated.

The lines joining these points are ideally straight lines (first order analysis). The Zimm-Extrapolation will be derived from extending the obtained function lines towards the axis and subsequently determining the appropriately extrapolated data point of the corresponding abscissa value of q^2 respectively $K \cdot c$. The hereby extrapolated values will be connected by a corresponding straight line and extended to the intersection point of the ordinate. The intercept value at the ordinate (y-axis) gives the inverse weight-average molecular weight ($1/M_W$). Additionally the Zimm-diagram provides the Radius of Gyration, derived from the slope of the extrapolation line $c \rightarrow 0$ respectively the second Virialcoefficient from the slope of the extrapolation line $q \rightarrow 0$.

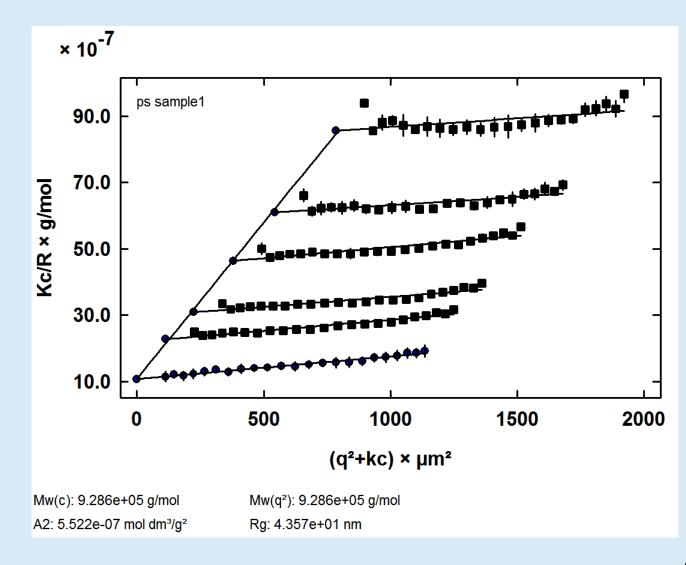
Berry-Plot (sqrt Kc/R vs. q²+kc)

- appropriate for samples with higher molecular weight , typ. diameter > 50nm

Guinier-Plot (In Kc/R vs. q²+kc)

- appropriate for small angles and large particles, $qR_g < 1$





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ALV / Static & Dynamic FIT and PLOT - [Ps_std1]		-
📘 File Edit Plot-Type q²-Dep. c-Dep. Plot-Options	Calculation Form-Factor:Pz	Window Help
🗁 🗶 🔓	Calculate	Strg+N
k: << >>	Results	Strg+R
117.1	Correction for Reflection	

via **Calculation** -> **Results** the result spread sheet can be obtained, in which molecular weight M_W , static structure factor <S²> and radius of gyration R_g for each concentrations as well as the extrapolated (c=0) concentration are plotted.

File	Conc/(g/dm³)	Mw(app)/(g/mol)	<s²>(app)/µm²</s²>	l l
Conc.=0	0.0000	9.286e5	1.898e-3	
Ps_std1.sta	6.7000	1.167e5	1.819e-4	
Ps_std1.sta	4.6400	1.641e5	2.447e-4	
Ps_std1.sta	3.2400	2.155e5	4.333e-4	
Ps_std1.sta	1.9100	3.237e5	5.819e-4	
Ps_std1.sta	0.9700	4.393e5	8.691e-4	

Results to Clipboard







Hard Sphere with Radius R

Spherical Capsule with outer radius R_0 and inner radius R_i

Cylinder with radius R and length h

 $R_g^2=rac{3}{5}R^2$

 $R_g^2 = rac{3}{5} rac{R_{
m o}^5 - R_{
m i}^5}{R_{
m o}^3 - R_{
m i}^3}$

Dependency of Gyrationsradius R for typical particle geometrics

$$R_g^2 = rac{R_{
m o}^2 + R_{
m i}^2}{2} + rac{h^2}{12}$$

 $R_q^2 = \frac{R^2}{2} + \frac{h^2}{12}$

Hollow Cylinder with outer radius R_{0} and inner radius R_i and length h

Ellipsoide

with semi-axis a, b and c

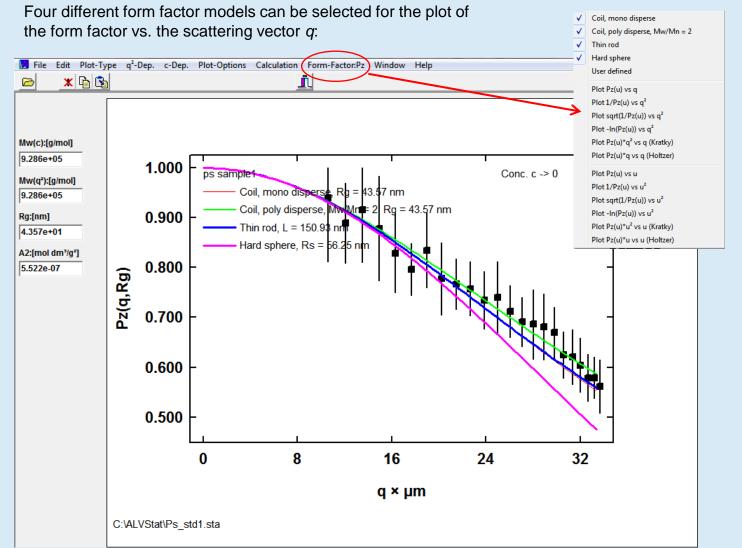
Disc-shaped Particle Flat disc with radius R

$$R_g^2 = rac{a^2 + b^2 + c^2}{5}$$

 $R_g^2 = rac{R^2}{2}$







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