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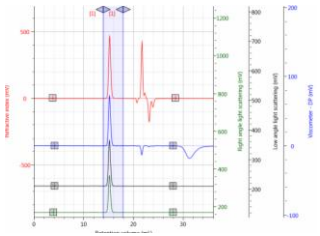
The OMNISEC™ Triple Detection method explained

SEC-LS User Training Course

Overview

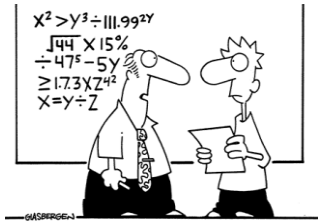
OMNISEC Training course – Tutorial 2

Why do we calibrate?



Steps in the Triple Detection method

Software Exercise 1



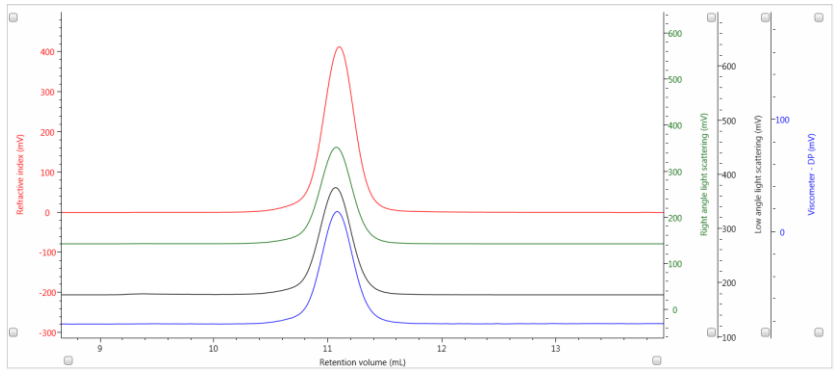
Discussion of results

Questions

How is the data calculated?

Analysis methods

How do we get from a SEC/GPC chromatograms to the results?



?

Results by sample and peak	
Parameter	Inj. 3 PS244893 108073 0...
	Peak 1
RV (mL)	16.97
Mn (g/mol)	107,400
Mw (g/mol)	247,100
Mz (g/mol)	433,700
Mw/Mn	2.3
IVw (dL/g)	0.8184
Rh(η)w (nm)	13.9
Rgw (nm)	16.55

Conventional calibration

Tutorial 4

Universal calibration

Tutorial 5

Triple detection method

Tutorial 1

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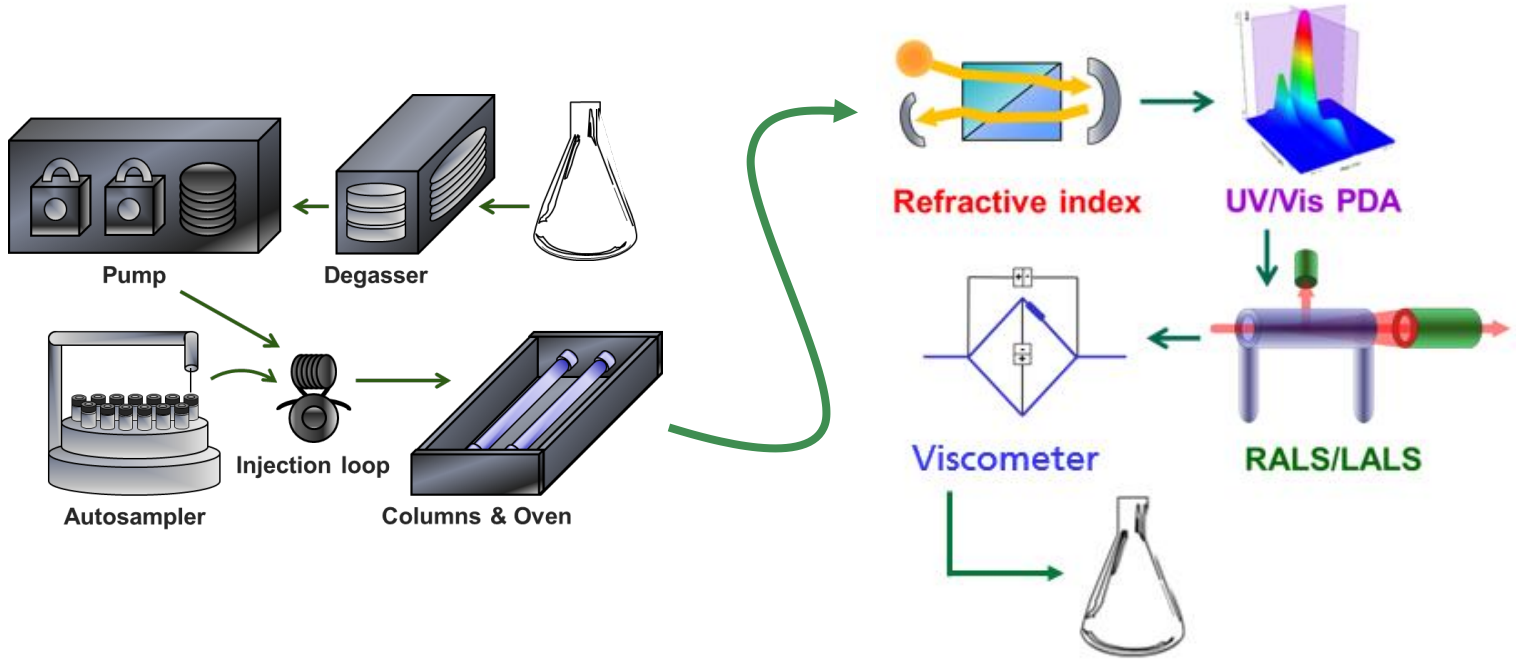
Hardware schematic

- **Triple Detection:** **Homopolymers – RI, LS, Viscometer**
- **Tetra Detection:** **Copolymers – RI, UV, LS, Viscometer**



GPC System and Detectors

OMNISEC

Premium Performance

RI- UV- RALS/LALS - Viscometer

- Redesigned RALS/LALS with significant sensitivity improvements
- New viscometer design
- Improved RI, including change of position in detector module, and excellent sensitivity

TDA max

Benchmark

UV – RALS/LALS – RI - Viscometer

SEC-MALS 20

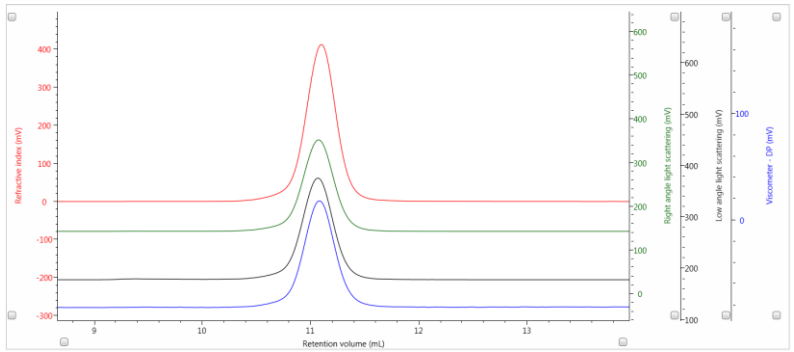
Modular detector

- Multi angle LS – 20 angles
- Absolute molecular weight of proteins, synthetic and natural polymers, as well as molecular size expressed as the radius of gyration, Rg.

Absolute molecular weight, molecular size, intrinsic viscosity, branching, conformation, protein aggregation.

Summary Triple Detection method

How do we get from a SEC/GPC chromatograms to the results?



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Run narrow and broad standards

Set baseline and limits

Create Calculation method (calibration)

Check Calculation method

Run samples

Set baseline and limits

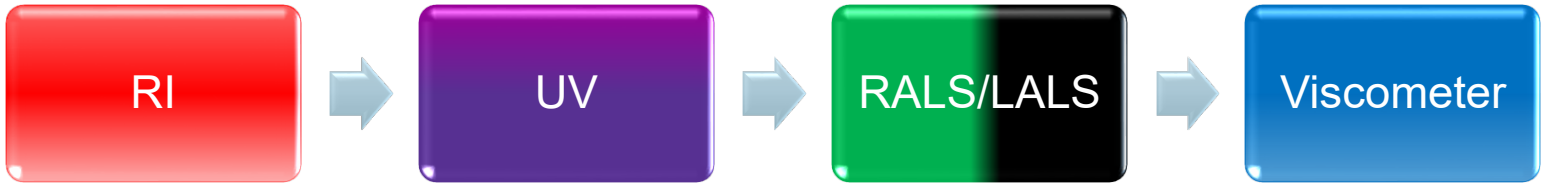
Obtain results

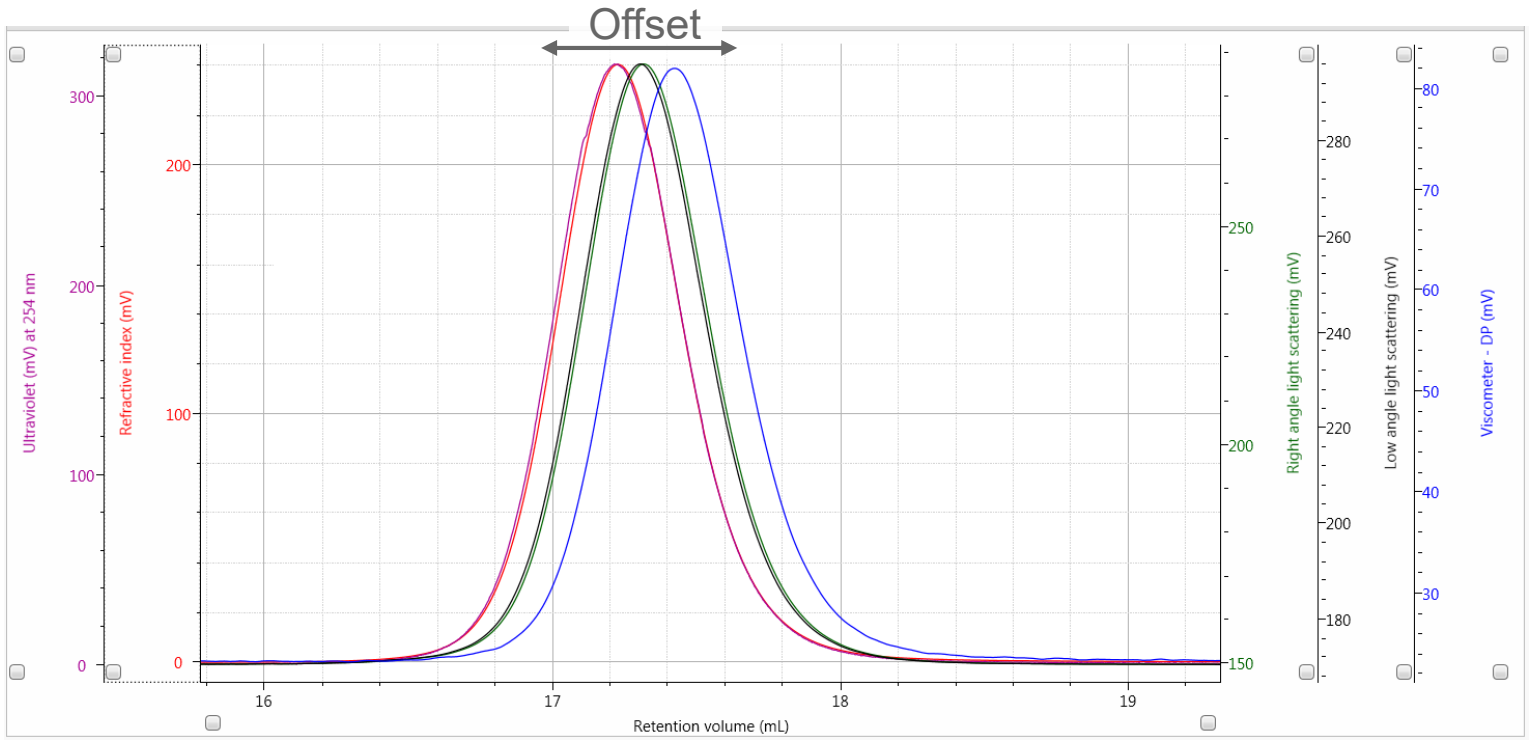
We will learn now why and how we do these steps.

Why do we calibrate?

1. Calculate detector offset
2. Determine peak symmetry and band broadening
3. Determine calibration constants

1 Calculate detector offset





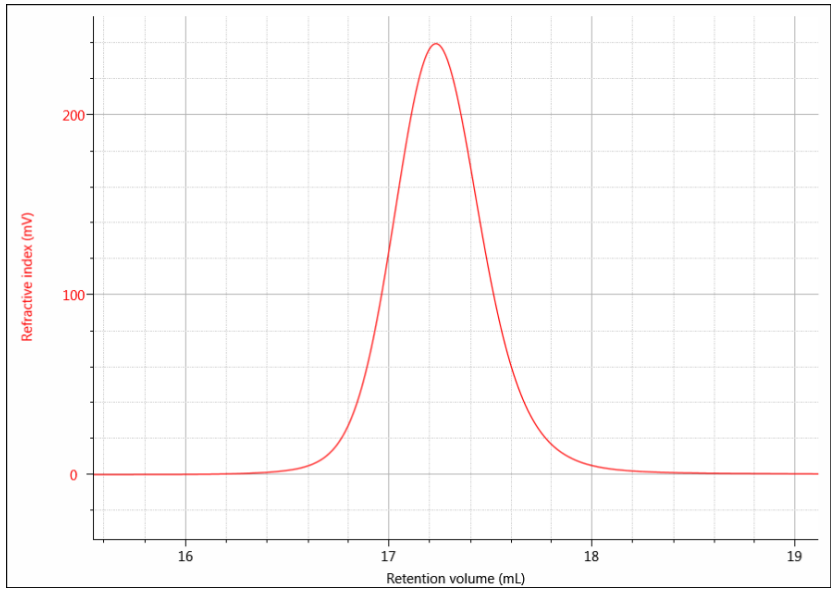
Detectors peaks offset is due to:

- the serial configuration in the OMNISEC
- the band broadening due to tubing

Software algorithms take into account these effects.

2 Determine peak symmetry and band broadening

The symmetry describes the shape of the peaks



Band-broadening looks into:

- The effect of running the sample through the chromatography system
- ‘stretching’ effect the sample is subjected to.

Equations Governing The Detectors

Concentration Detectors

Differential Refractive Index

$$RI_i = \frac{K}{n_0} \cdot \frac{dn}{dc} \cdot C_i$$

UV-Vis

$$A_i = K \cdot \frac{dA}{dc} \cdot C_i$$

Hydrodynamic Size and Intrinsic Viscosity

Four-capillary viscometer

Intrinsic Viscosity

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \frac{4\Delta P}{P_i - 2\Delta P} = [\eta] \cdot C$$

Absolute Molecular Weight Measurement

Static Light Scattering

Right Angle (90°) and Low Angle (7°) Light Scattering

$$\frac{K \cdot \left(\frac{dn}{dc}\right)^2 \cdot C}{R_\theta} = \frac{1}{M_w P(\theta)} + 2A_2C + 3A_3C^2 + \cdots$$

3 Determine calibration constants

Equations governing the detectors

$$RI\ output\ (mV) = K_{RI} \cdot dn/dc \cdot concentration$$

$$UV\ output\ (mV) = K_{UV} \cdot dA/dc \cdot concentration$$

$$Visc.output\ (mV) = K_{Visc.} \cdot IV \cdot concentration$$

$$LS\ output\ (mV) = K_{LS} \cdot M_w \cdot (dn/dc)^2 \cdot concentration$$

Unknown

By running the narrow standard of known concentration, dn/dc and M_w the detectors constants are calculated

$$K_{RI} = \frac{RI\ output\ (mV)}{dn/dc \cdot concentration}$$

$$K_{UV} = \frac{UV\ output\ (mV)}{dA/dc \cdot concentration}$$

$$K_{Visc.} = \frac{Visc.output\ (mV)}{IV \cdot concentration}.$$

$$K_{LS} = \frac{LS\ output\ (mV)}{M_w \cdot (dn/dc)^2 \cdot concentration}.$$

Triple Detection method

Step-by-step to obtain the M_w of your samples

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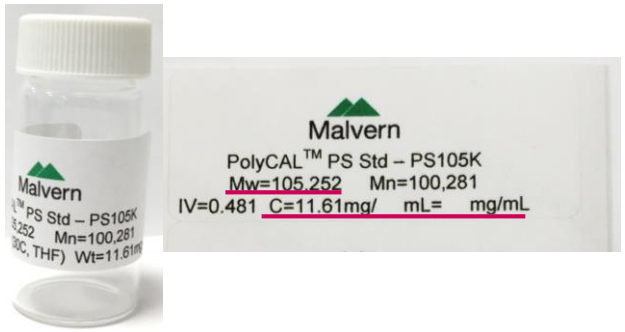
Calibration with narrow standard (e.g. PS105k in THF)



PS in THF – dn/dc 0.185 mL/g

$$K_{RI} = \frac{RI\ output\ (mV)}{dn/dc \cdot concentration}$$

$$K_{LS} = \frac{LS\ output\ (mV)}{M_w \cdot (dn/dc)^2 \cdot concentration}$$

Samples analysis – determine your sample M_w

Sample X
M_w - ?
Conc. – sample weighed and prepared accurately

Sample X in THF – dn/dc ? mL/g

$$dn/dc = \frac{RI\ output\ (mV)}{K_{RI} \cdot concentration}$$

$$M_w = \frac{LS\ output\ (mV)}{K_{LS} \cdot (dn/dc)^2 \cdot concentration}$$

Steps in the Triple Detection method?

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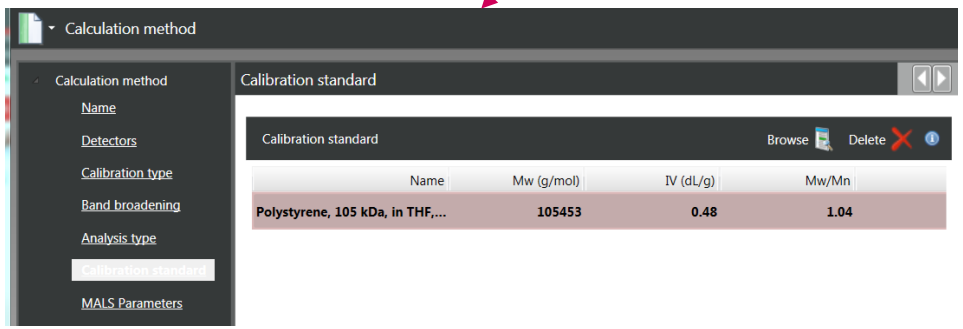
Define regions of valid signal in chromatogram

Define standard parameters

Determine detector constants

Determine sample parameters

Set baselines and limits

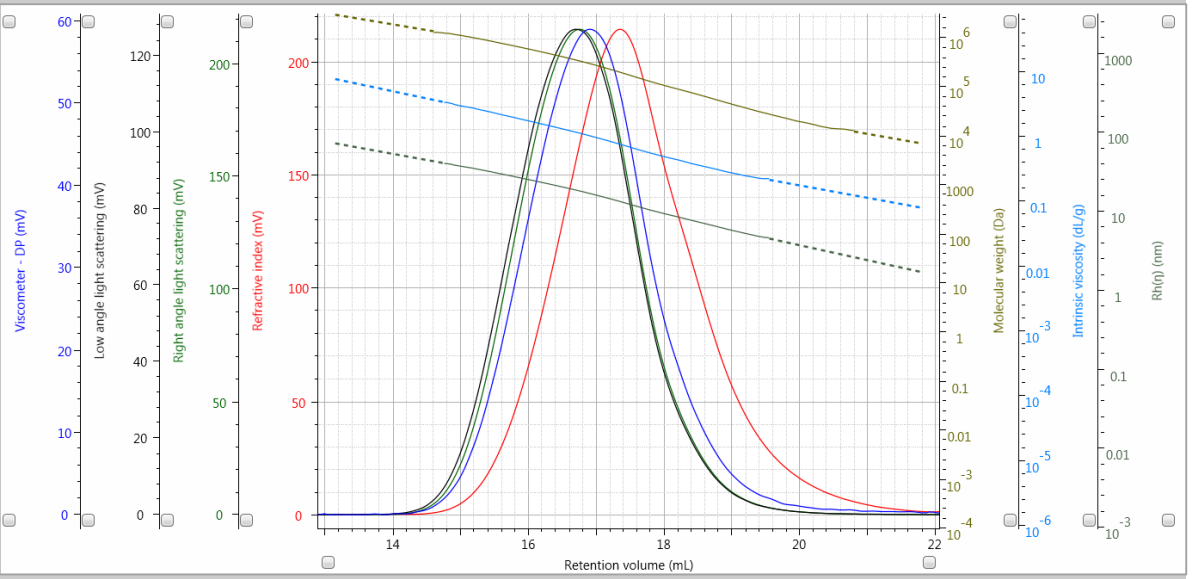


Detectors

Active	Detector	Detector constant	Offset (mL)
☑	RI	9,111,000	0
☐	UV	1,602,000	0
☑	RALS	3.513E-8	0.1267
☑	LALS	3.699E-8	0.1267
☑	DP	1.046	0.21
	IP	0	0.21

Results by sample and peak

Parameter	Inj. 1 PS 244893 108073...
	Peak 1
RV (mL)	16.68
Mn (g/mol)	98,270
Mw (g/mol)	248,100
Mz (g/mol)	446,700
Mw/Mn	2.524
IVw (dL/g)	0.7945
Rh(η)w (nm)	13.74
Rgw (nm)	11.84
M-H a	0.6549
M-H log K (dL/g)	-3.602
RI peak (mV·mL)	290.1
RALS peak (mV·mL)	347.7
LALS peak (mV·mL)	349.9
DP peak (mV·mL)	119.9
MALS peak (mV·mL)	N/C
Calc. dn/dc	N/C
Recovery (%)	100.3



Results by sample and peak	
Parameter	Inj. 1 PS245k Mw 245245...
	Peak 1
RV (mL)	17.36
Mn (g/mol)	103,700
Mw (g/mol)	235,500
Mz (g/mol)	394,100
Mw/Mn	2.271
IVw (dL/g)	0.8220
Rh(η)w (nm)	13.72
Rgw (nm)	N/C
M-H a	0.6992
M-H log K (dL/g)	-3.817

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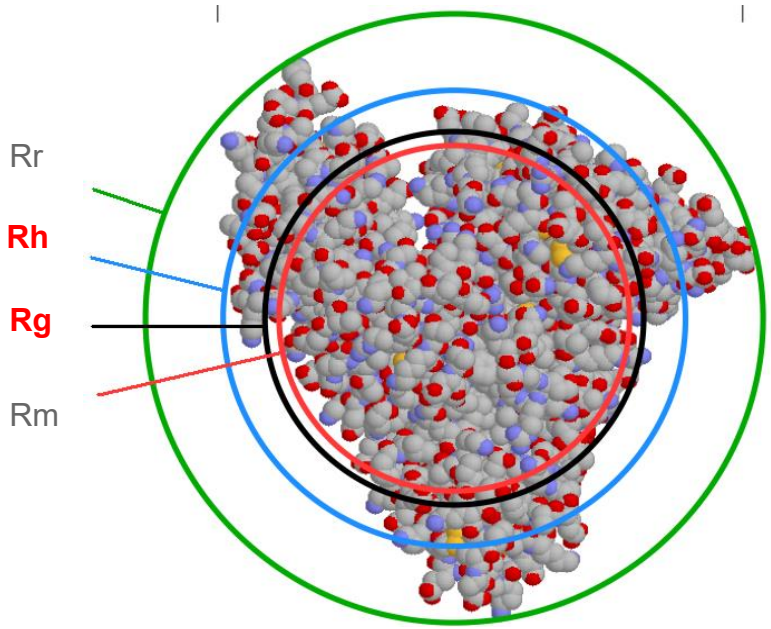
Triple/Tetra Detection GPC/SEC Results

- Absolute molecular weight
- Molecular weight distribution
- Intrinsic viscosity
- Molecular size
- Mark-Houwink coefficients
- % Polymer

Molecular size by triple detection

(Random Coil Polymer)

- Two different measurements of size can be performed using Triple Detection GPC
- Hydrodynamic Radius (R_h)
  - Viscometer and light scattering data
- Radius of Gyration (R_g)
  - Comparison of light scattering detector intensities
  - Flory-Fox estimation



Size measurements - R_h

Hydrodynamic Radius (R_h)

Triple Detection SEC/GPC – IV and M_w

R_h is the radius of an equivalent solid sphere that increases the fluid viscosity by the same amount as the macromolecule.

$$[\eta]M = \frac{10}{3}\pi \cdot N_A \cdot R_h^3$$

IV M_w Constants

Triple Detection

- Analyze hydrodynamic size from < 1 nm to the exclusion limit of the SEC column (~200 nm)
- No extrapolation or fitting parameters

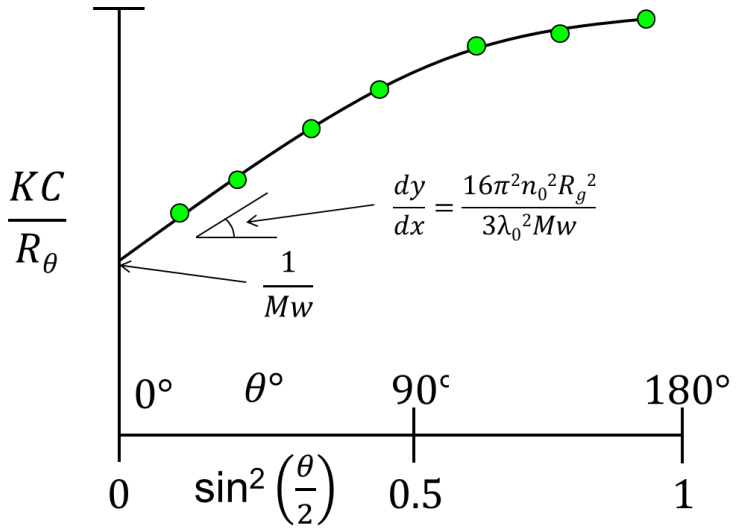
Dynamic Light Scattering (DLS) – Zetasizer products

R_h is the radius of an equivalent sphere that diffuses with the same speed as the molecule of interest.

Size measurements - R_g

Radius of Gyration (R_g)

Rg is the root-mean-square of the radii from the centre of the mass to the different mass cores within the molecule.



- Direct measurement by changes in scattered light intensities with observation angle
  - RALS/LALS
  - MALS

Limitations:

- Requires good S/N light scattering signal
- Lower size detection limit = 10-15 nm
  - Limit of Anisotropic scattering
- Large structures require non-linear curve fitting

Size measurements - R_g

Radius of Gyration (R_g)

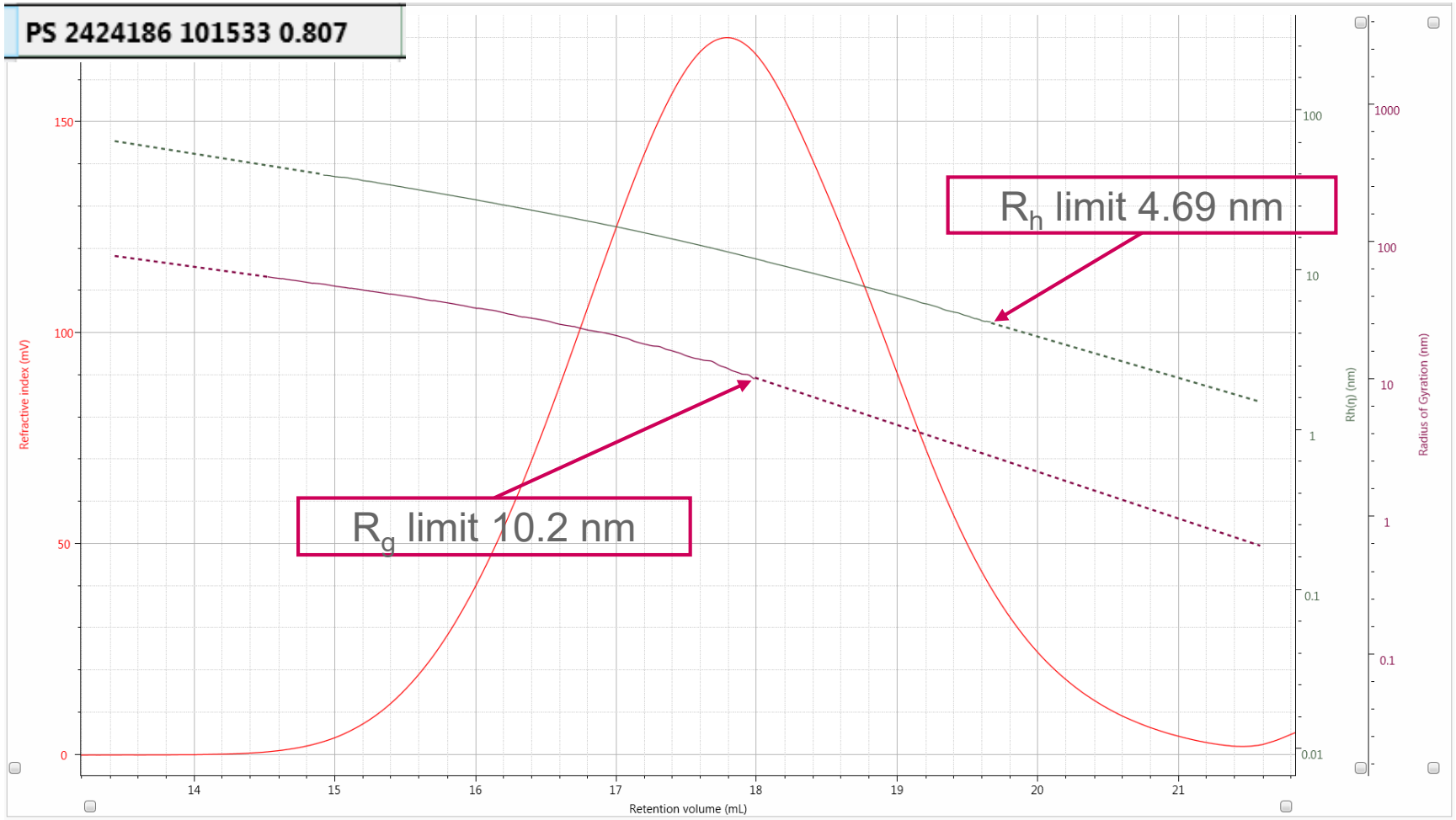
A good estimate of R_g can be made using viscometry and the Flory-Fox equation

$$R_g = \frac{1}{\sqrt{6}}\left(\frac{[\eta]M_w}{\Phi}\right)^{\frac{1}{3}}$$

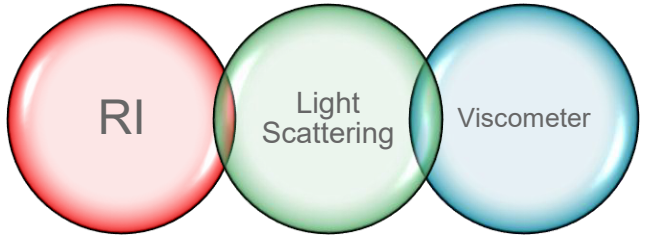
Equation relates the intrinsic viscosity of a flexible coil molecule in solution in terms of R_g

- Where **Φ** = Florys universal constant
- Only OmniSEC v5

Comparison of R_h and R_g distribution for broad polystyrene 235k



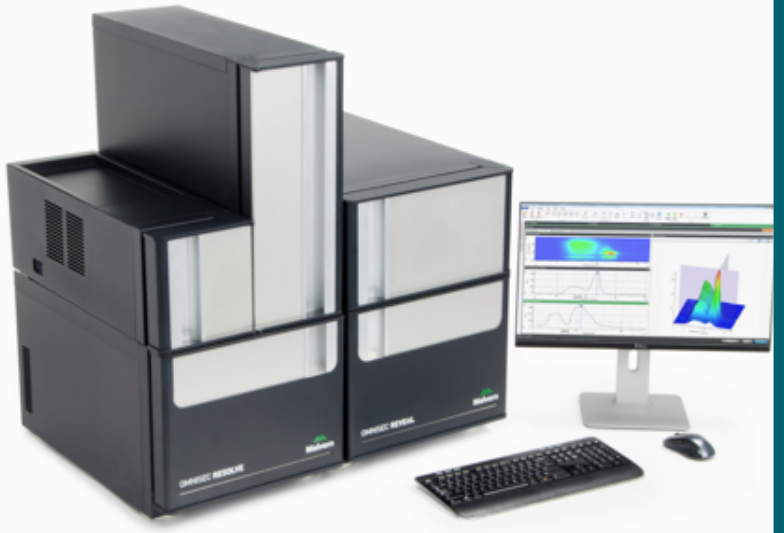
- Triple detection technique uses 3 detectors:



- Specific equations govern each detector which allow
  - concentration
  - M_w
  - intrinsic viscosity
  - molecular size

  to be determined across the entire distribution.
- Adding a fourth detector – UV – allows for <u>compositional analysis</u> in copolymer samples.

Questions?



Summary

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Fale comigo!

Gustavo G. Parra, PhD

Cel: +55 11 94556-5914

E-mail: gustavo.parra@pensabio.com.br

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