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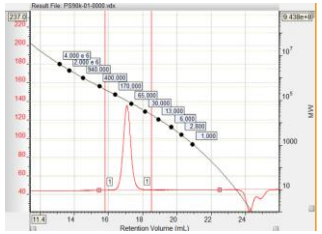
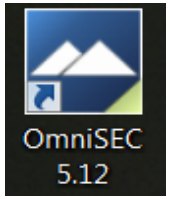
Conventional calibration

SEC User Training Course

Overview

OMNISEC Training course – Tutorial 4





Why, when and how!

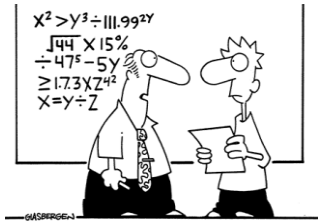
Hardware schematic

Detector schematic

Steps in conventional calibration

Limitations

Software Exercise 3



Discussion of results

Questions

Why, when and how?

- Relatively low Cost
- Easy to use: Pump + Column + Detector
- Conventional used with one Concentration Detector
  - RI (Almost Universal)
  - UV (Second Most Used – Proteins & UV Active)
- Publications usually state Mw '*relative to*' or indicate the use of multiple standards
  - (i.e. range from ~1000Da – 4MDa)
- Column Retention Volume must be known
  - Remember: Separation by Size, not Molecular Weight

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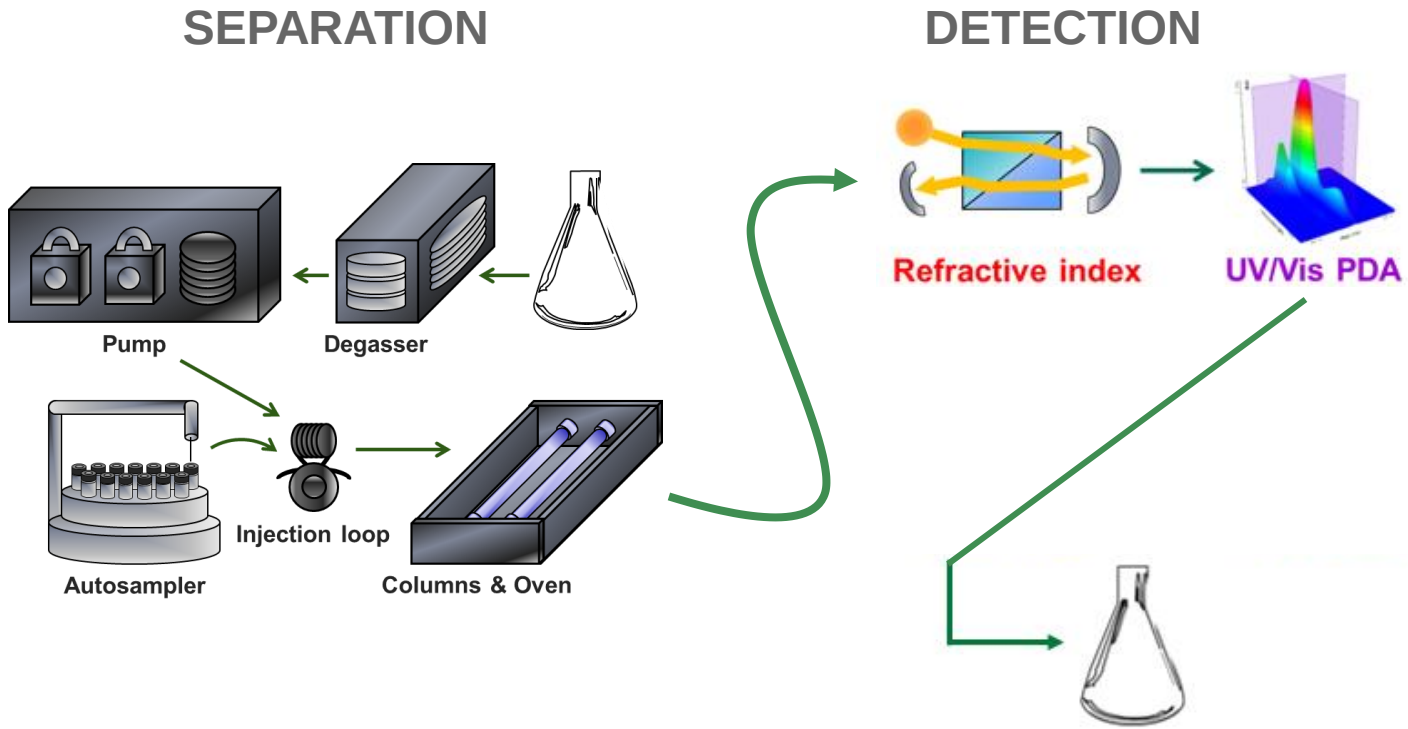
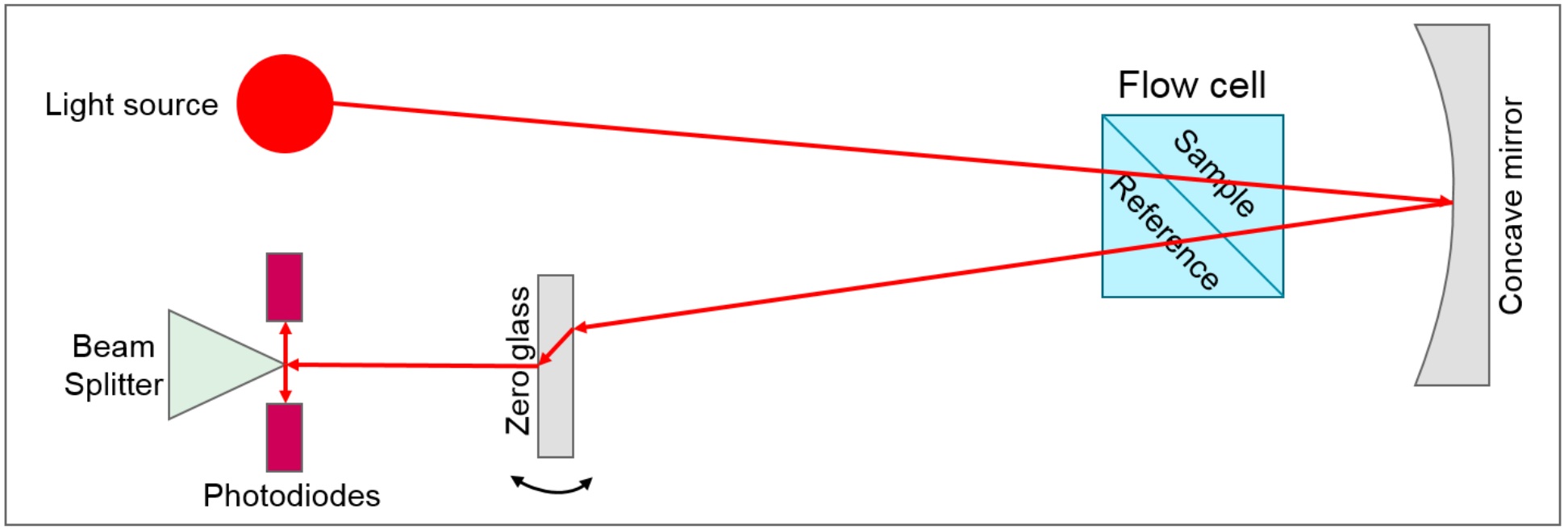
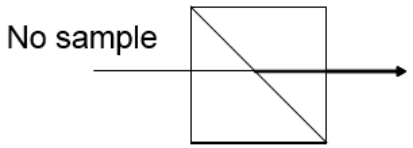
Hardware schematic

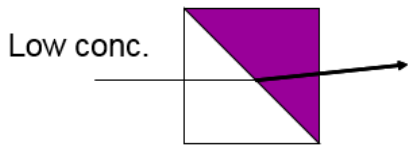
Conventional calibration system

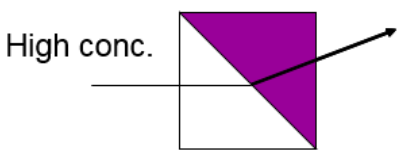


Detection - RI detector schematic







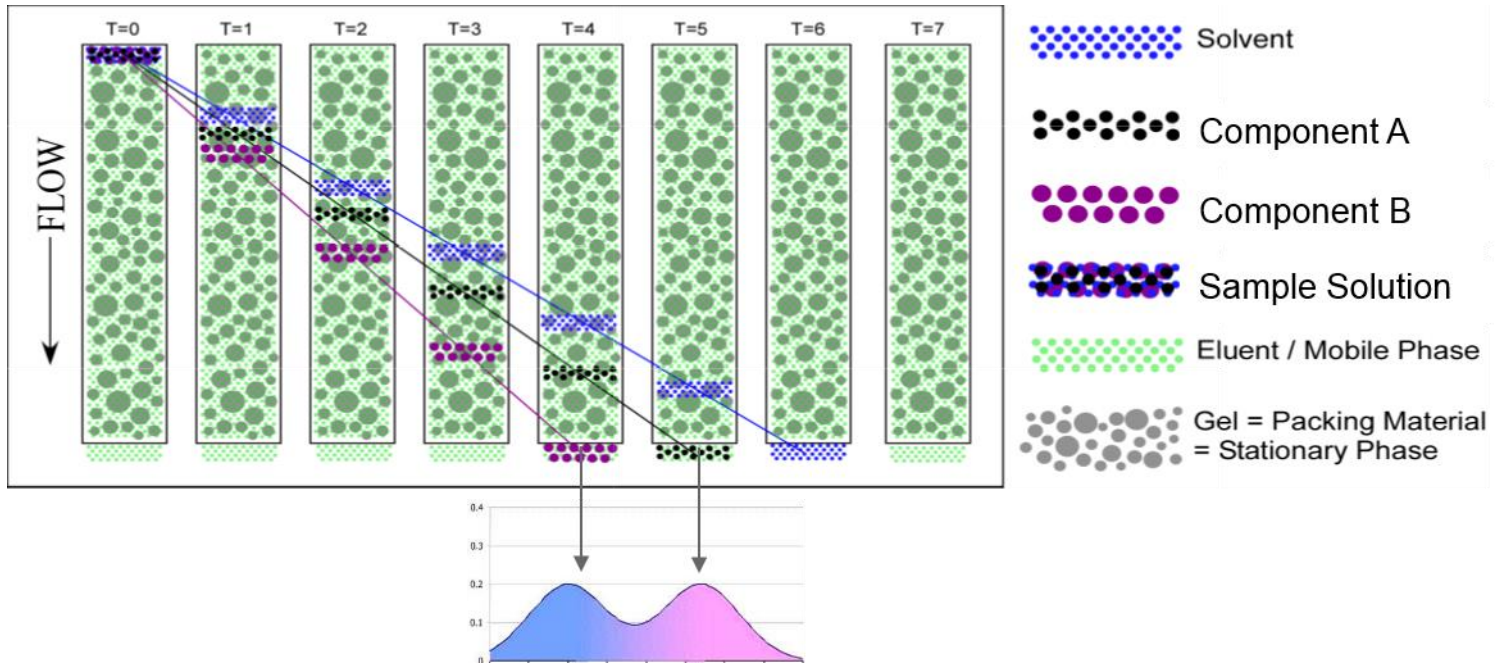


Differential refraction between solvent and solution results in different signals at the photodiodes.

SEC/GPC - Separation

GPC (also known as size-exclusion chromatography, SEC) has long been used as a key tool for measuring molecular weight

- GPC separates macromolecules in solution according to size in a chromatographic column
- After the column, the separated molecules can be analysed by one or more detectors



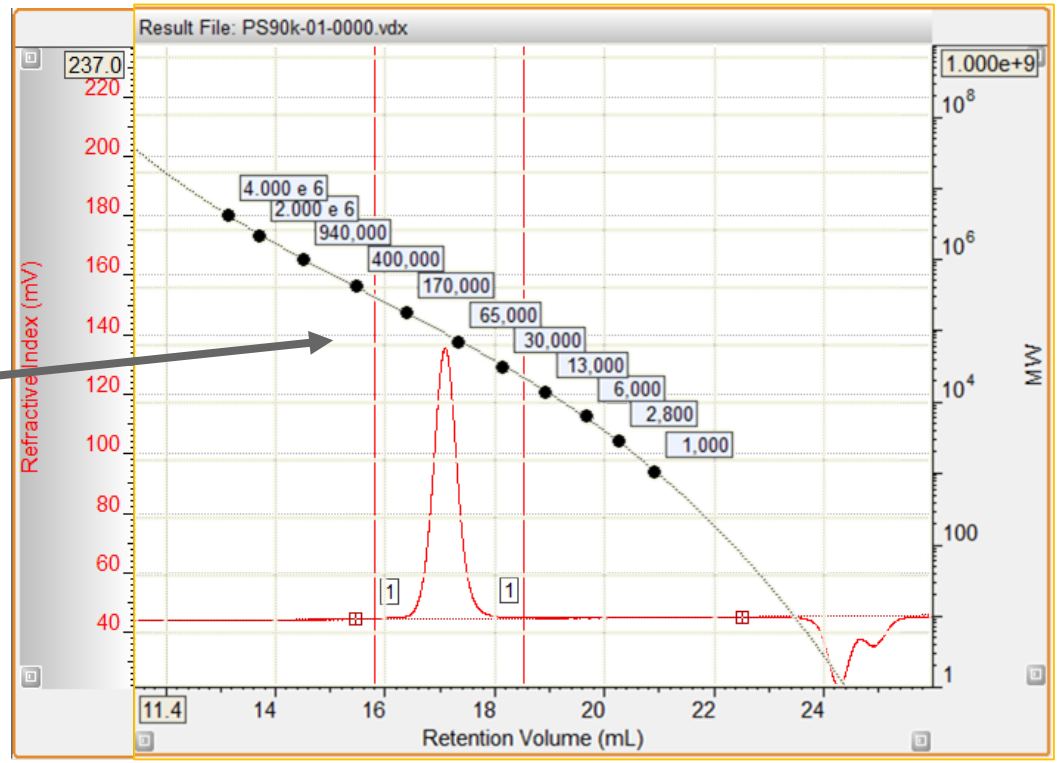
Conventional calibration outline

Conventional GPC is the most widely used calculation method

Concentration detector: **RI** or **UV**

Series of **standards** – conventional SEC/GPC

Relative Molecular weight

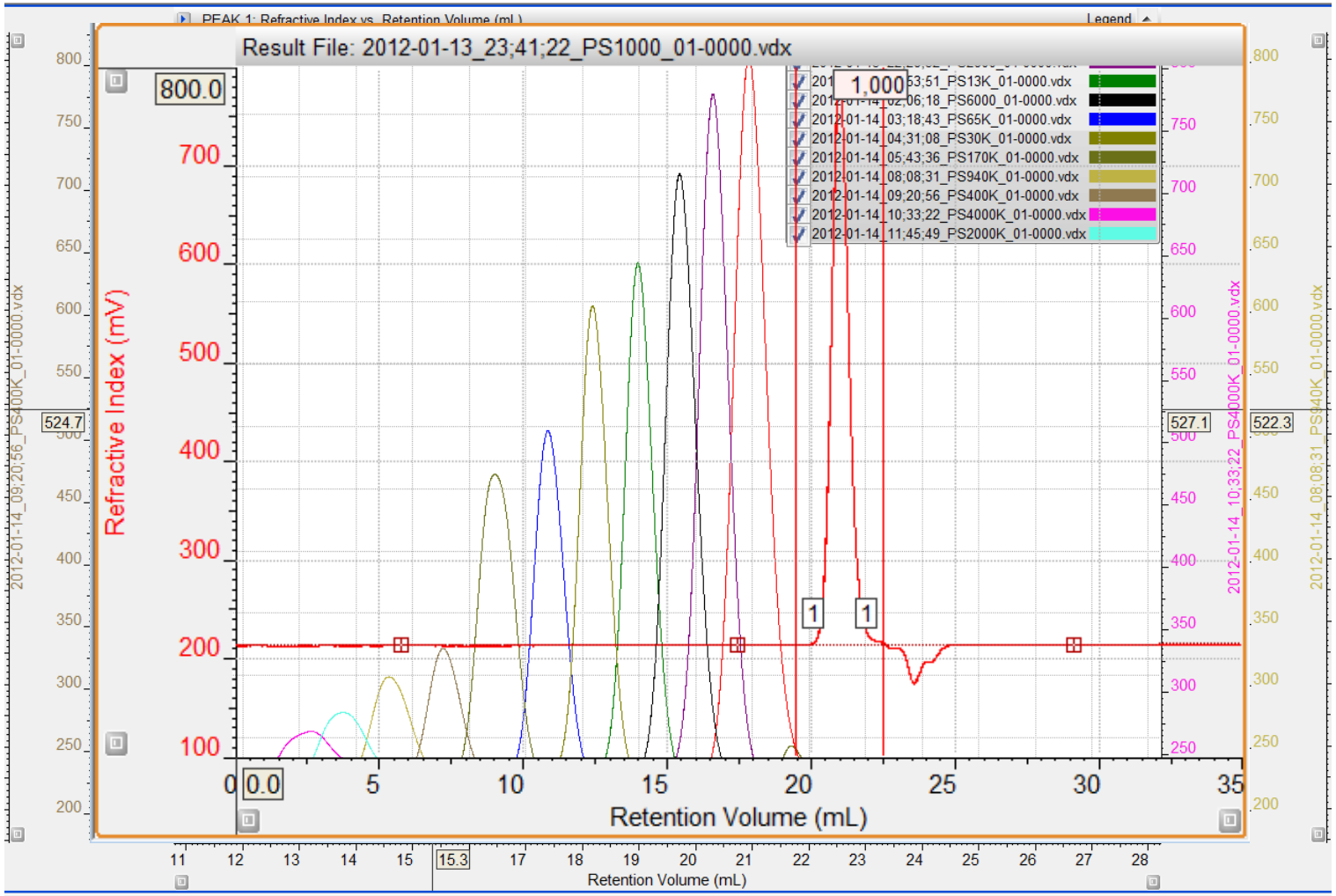


- To measure an unknown sample, the column retention volume must be calibrated in some way
- Use polymer standards of known molecular weight
- Flow rate must be controlled carefully
- Accurate concentration not necessary

Remember: the columns separate by size not molecular weight so the calibration is only relative

1st step: run standards and create calibration curve

A series of standards – 10 to 12 standards

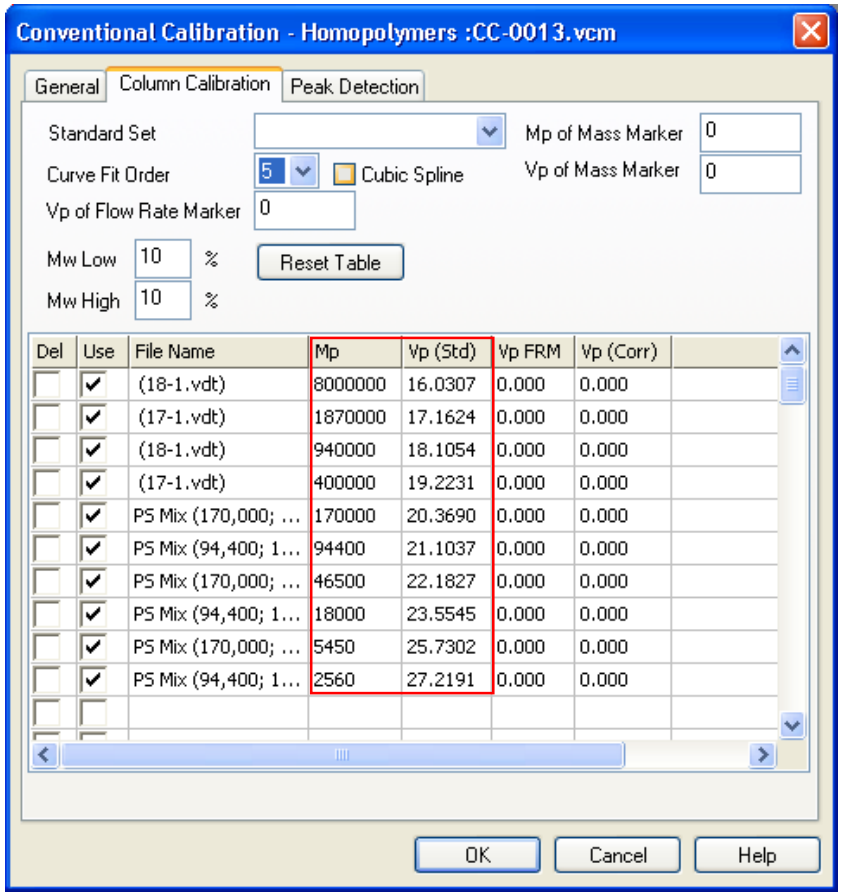


- Series of standards with a range of molecular weights
- Set baselines and limits around each standard to perform calibration (Software exercise 3)

1st step: run standards and create calibration curve

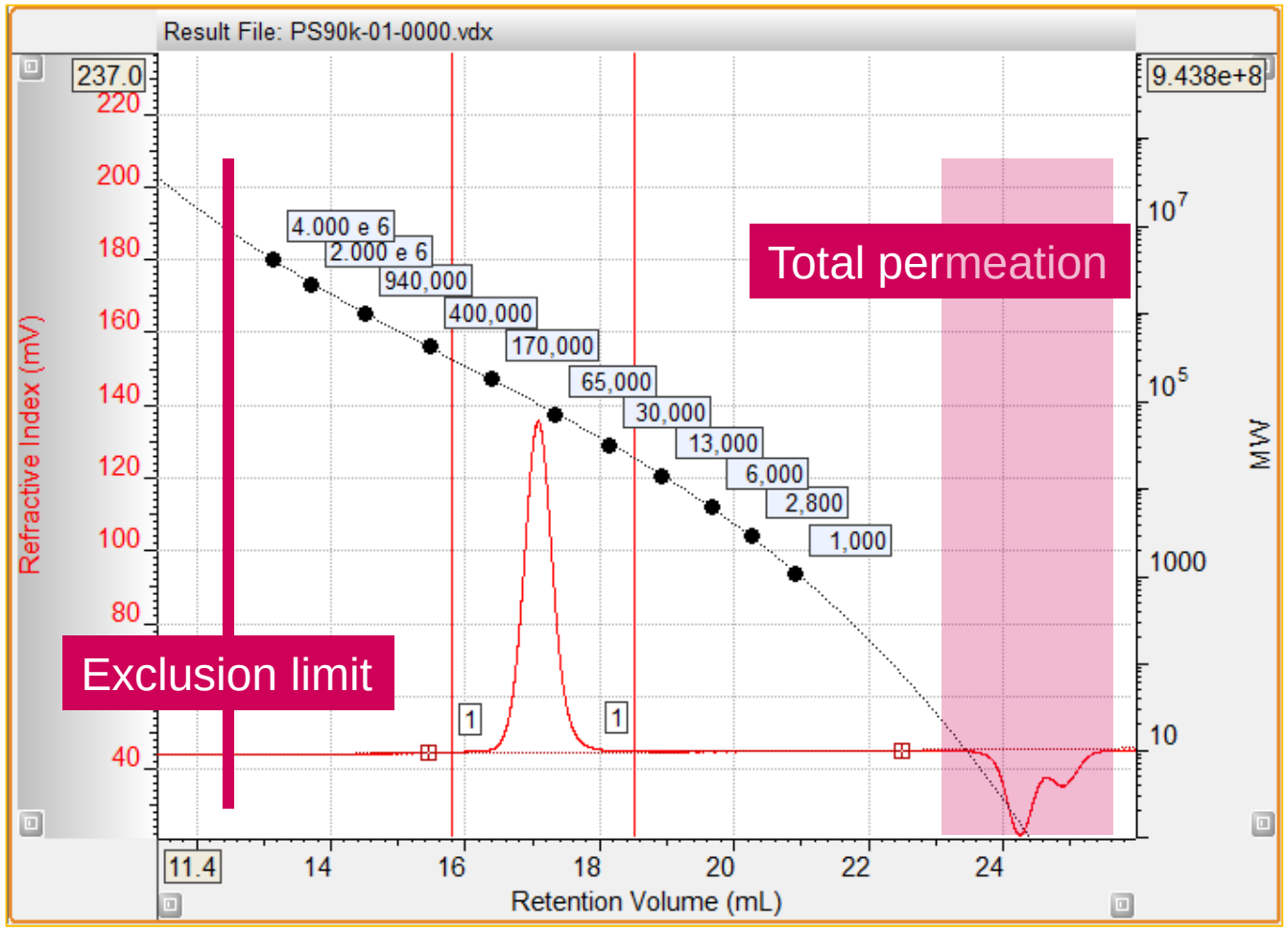
Narrow standards calibration

- Table of Standards on OmniSec V. 5
  - M_p and Vp: molecular weight and retention volume at the peak
  - Software exercise 3 – conventional calibration



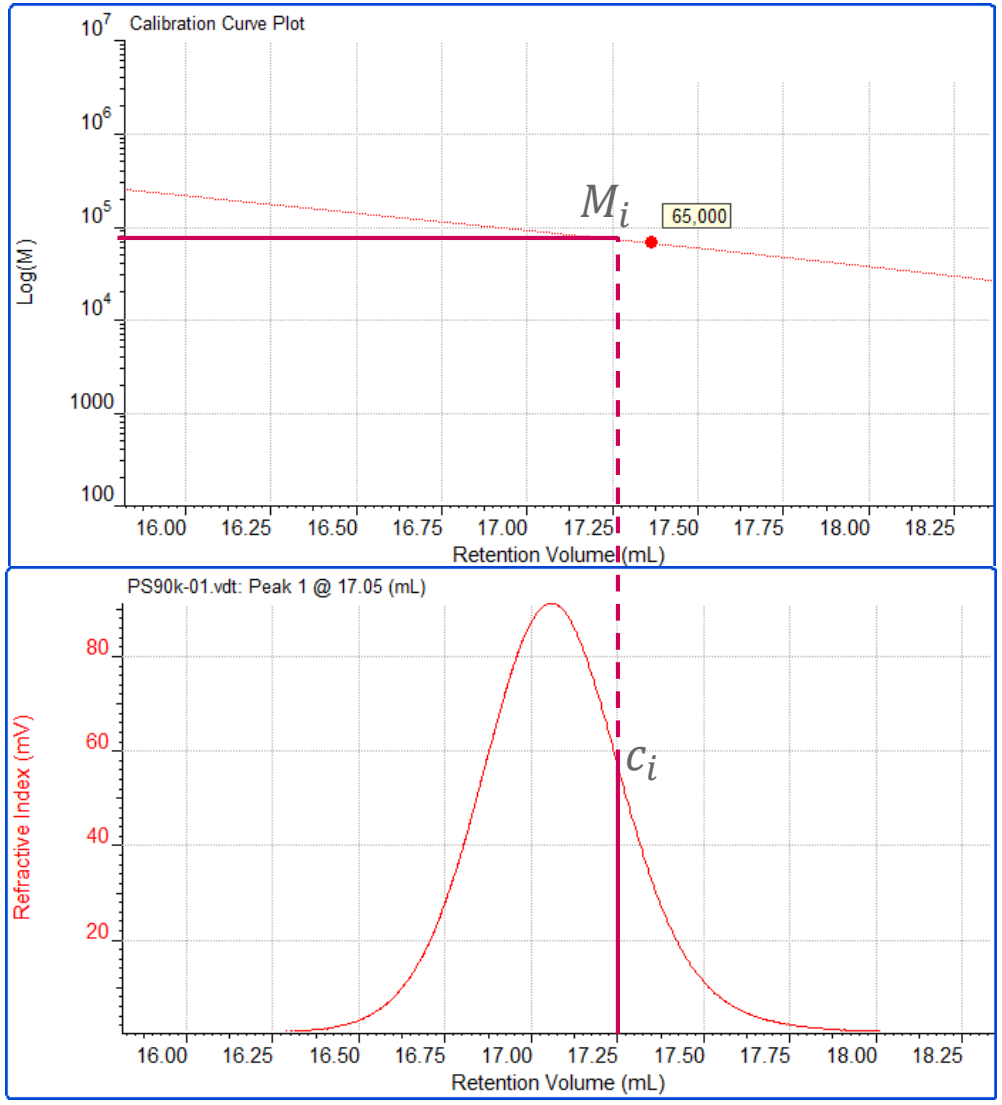
2nd step: run unknown sample

Calibration line and calculation



- Total permeation
  - defines the point at which everything that was injected has passed through the column
- Exclusion limit
  - defines the maximum size of a molecule that can be separated by a column

How the M_w is calculated in conventional calibration



Molecular weight moments

- Number Average (M_n)

$$\overline{M_n} = \frac{\sum c_i}{\sum {c_i}/{M_i}}$$

- Weight average (M_w)

$$\overline{M_w} = \frac{\sum c_i M_i}{\sum c_i}$$

- Z-average (M_z)

$$\overline{M_z} = \frac{\sum c_i M_i^2}{\sum c_i M_i}$$

How the M_w is calculated in conventional calibration

Molecular weight moments

M_n = Total mass of material divided by the total number of molecules.

- Mid point of the distribution in terms of numbers of molecules
- Sensitive to low MW species (more molecules in a given mass)

M_w = Multiplying by the molecules mass.

- Weights each chain length according to its weight fraction.
- Mid point of the distribution in terms of polymer weight
- Biased towards larger molecules in the distribution

M_z = Multiplying by the molecules mass again.

- Heavily weighed towards the largest molecules in the sample.
- Sedimentation properties

- Number Average (M_n)

$$\overline{M_n} = \frac{\sum c_i}{\sum {c_i}/{M_i}}$$

- Weight average (M_w)

$$\overline{M_w} = \frac{\sum c_i M_i}{\sum c_i}$$

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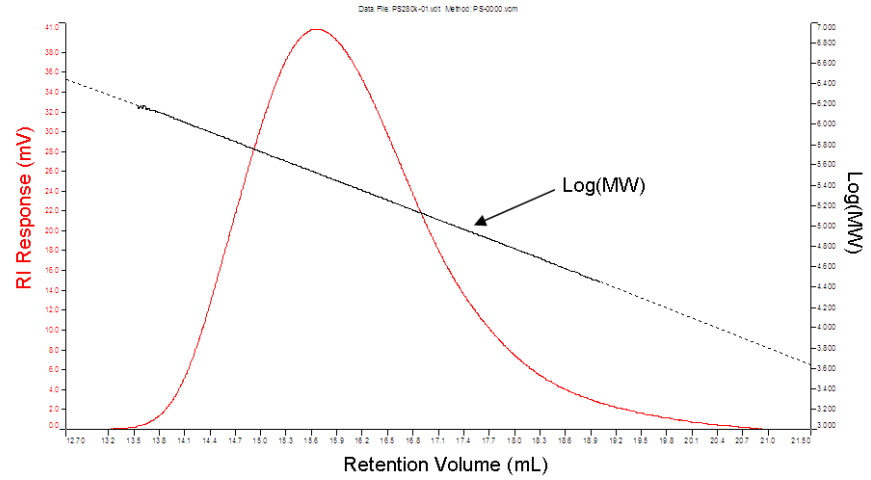
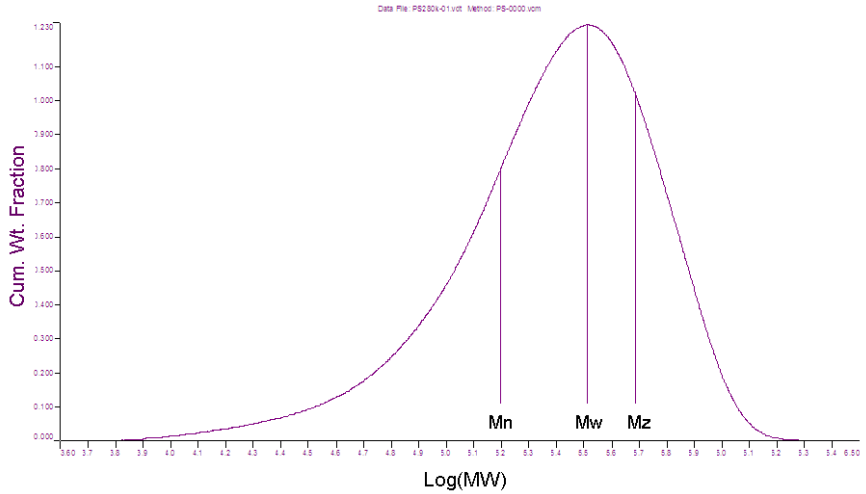
How the M_w is calculated in conventional calibration

Molecular weight moments





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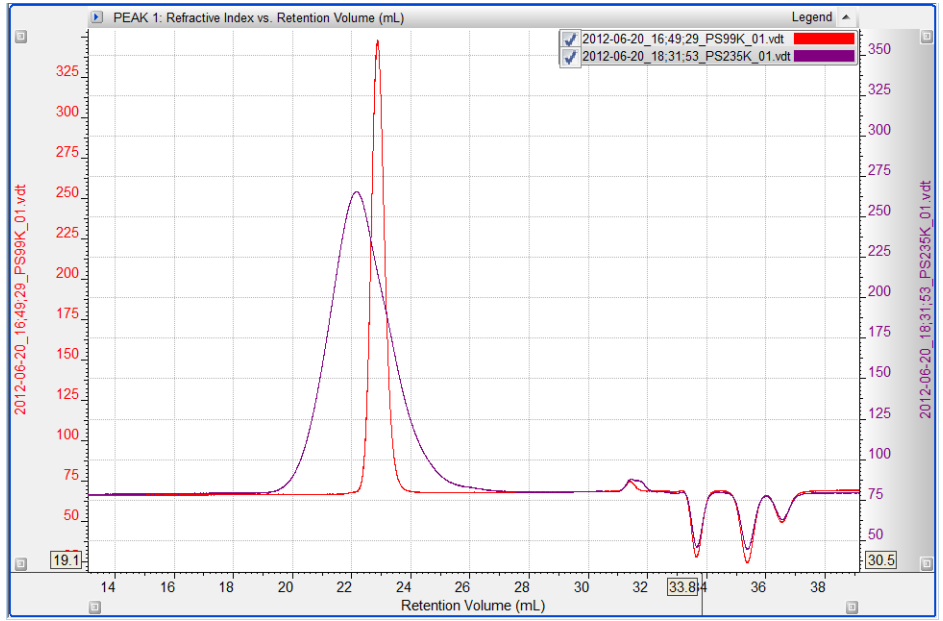
Dispersity Đ

Classification of molecular weight distribution

Type of material	Đ = M_w/M_n
Monodisperse	= 1.0
Narrow distribution	< 1.2
Medium distribution	< 2.0
Broad distribution	> 2.0

Dispersity

$$Đ = \frac{M_w}{M_n}$$



Narrow distribution

Đ < 1.2

Broad distribution

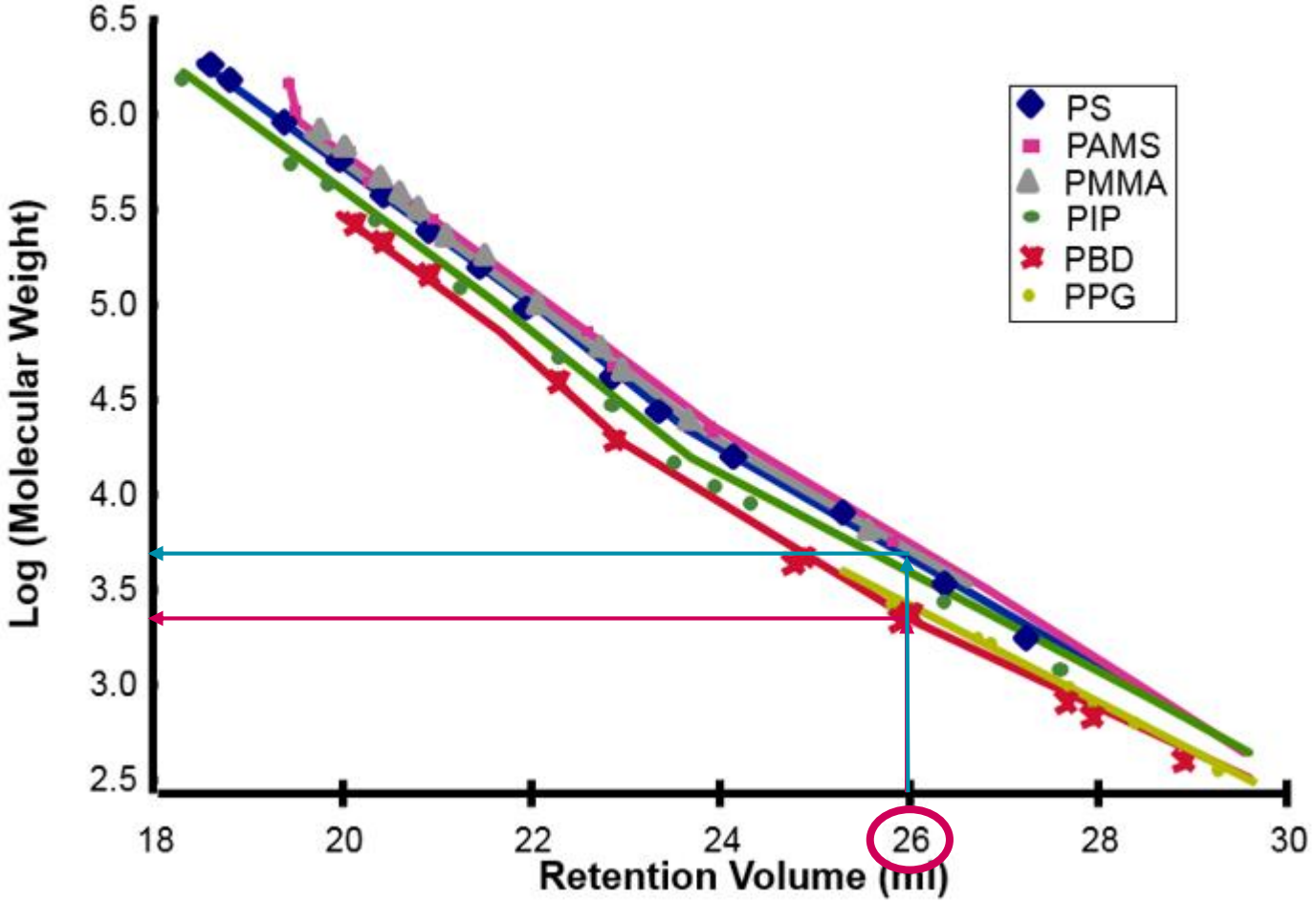
Đ > 2.0

Advantages of conventional calibration

- Simple setup
  - *Only one detector required – RI or UV*
- Accurately known concentrations not critical for technique
  - *Approximate concentrations are good enough*
- As a technique – excellent precision (repeatability)
  - *Dependent on column and pump performance*

So… where is the disadvantage with conventional calibration?

Overlay of conventional calibration curves



Sample eluting at ret. vol. of 26 ml:

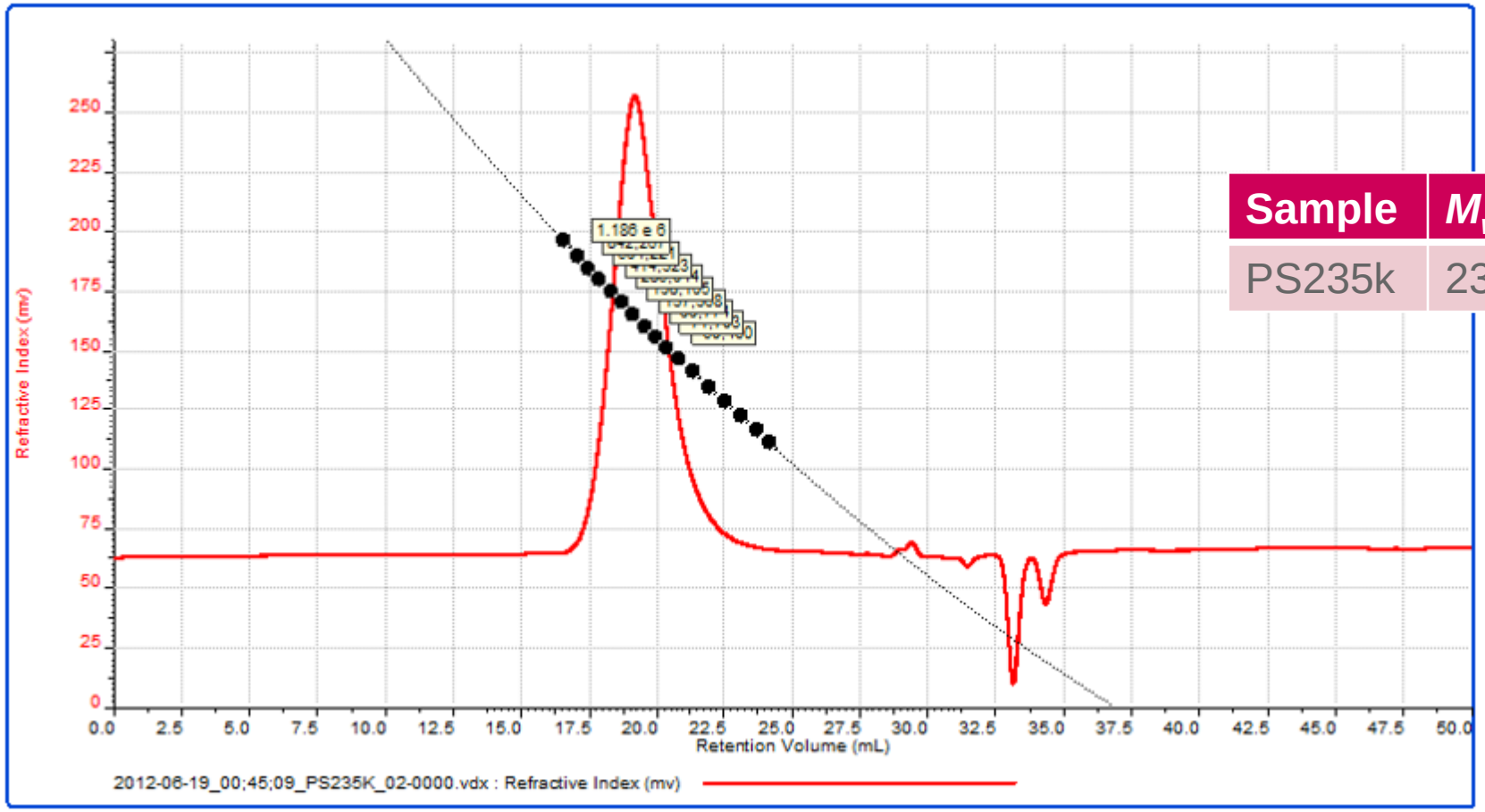
1) PBd calibration curve
   - Log(M_w) = 3.4
2) PS calibration curve
   - Log(M_w) = 3.6

Each polymer has its own size to molecular weight relationship:

$$V_h \approx [\eta] \cdot M$$

Conventional calibration of polymers with the same chemistry

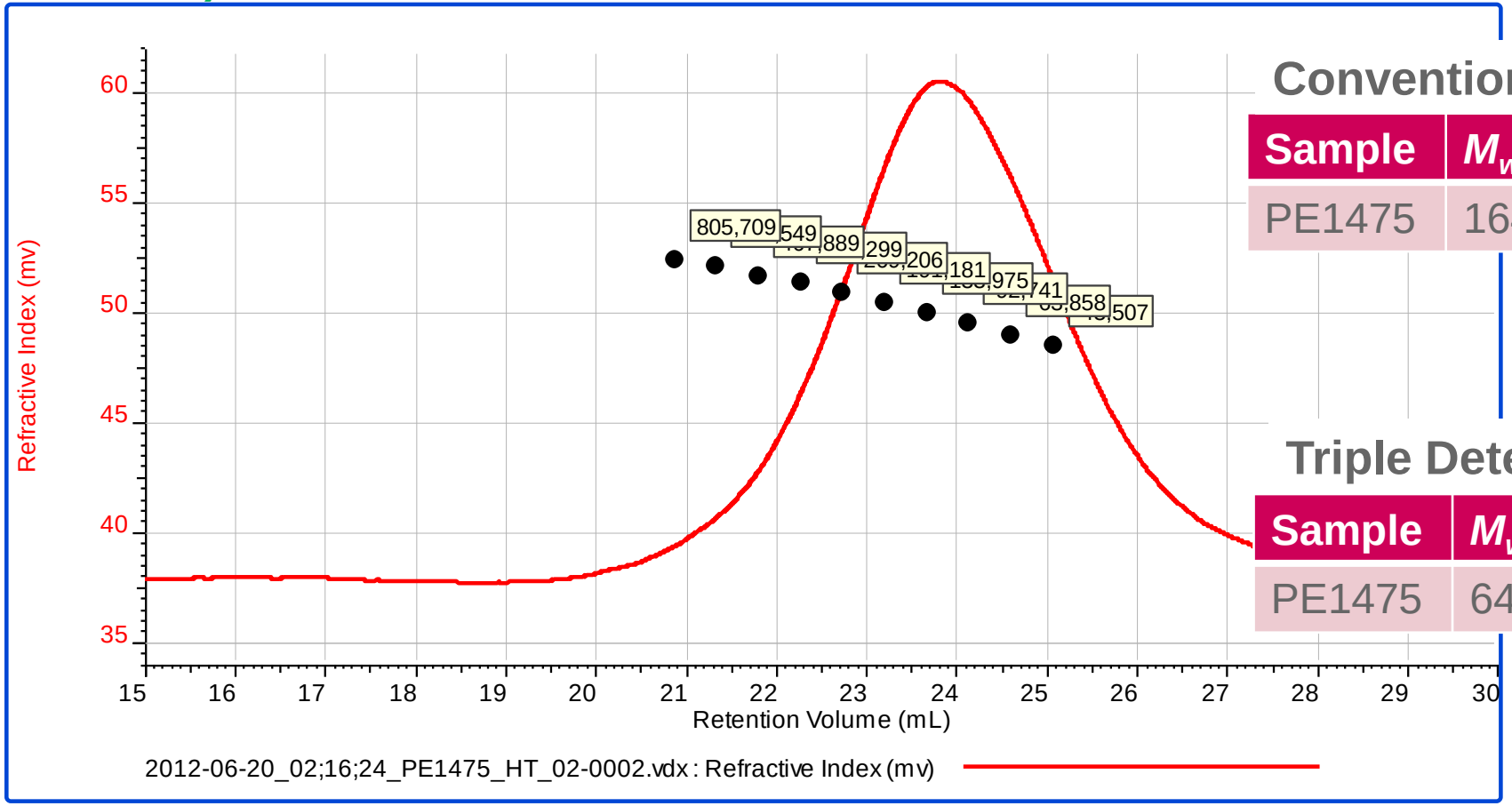
Polystyrene (sample) relative to polystyrene (calibration standards)



Sample	M_w	M_n	M_w/M_n
PS235k	231,974	88,527	2.620

Conventional calibration of polymers with different chemistry

Polyethylene (sample) relative to polystyrene (calibration standards)



Conventional calibration results

Sample	M_w	M_n	M_w/M_n
PE1475	164,733	20,326	8.105

Triple Detection method results

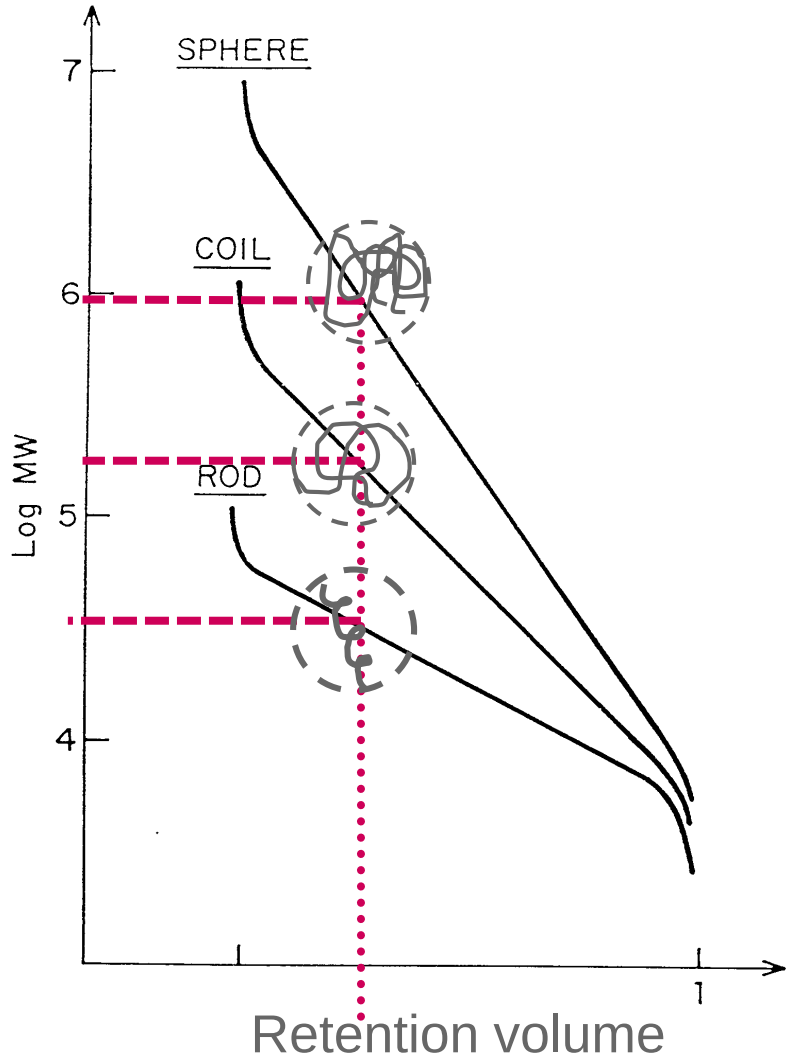
Sample	M_w	M_n	M_w/M_n
PE1475	64,129	16,782	3.821

Effect of molecular shape on GPC retention volume

Log $M_w \approx 6$

Log $M_w \approx 5.3$

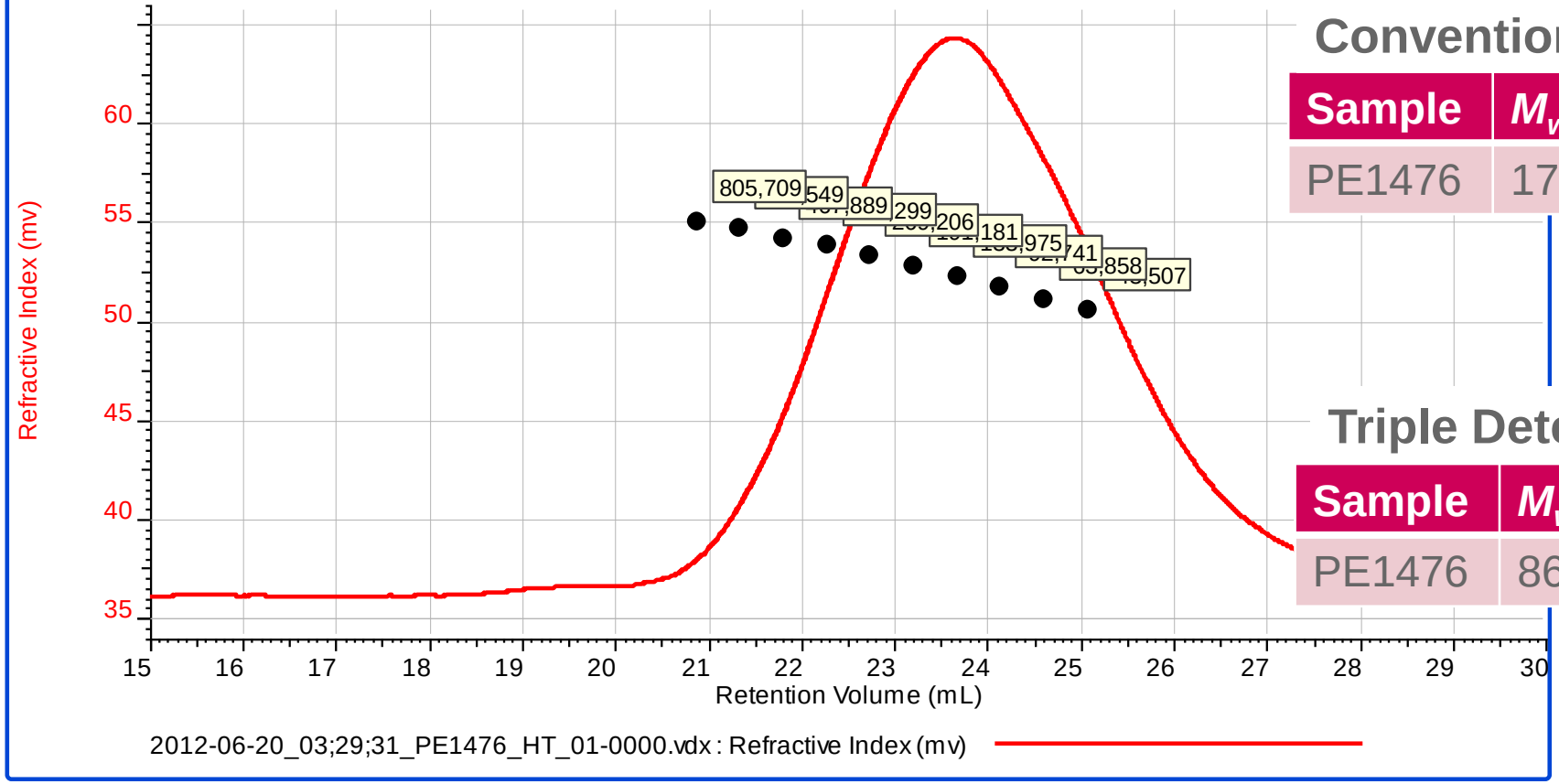
Log $M_w \approx 4.5$



- Size exclusion columns separate by hydrodynamic size and not by M_w
- Therefore, structural differences will affect results:
  - **Conformation**
  - **Branching**

Conventional calibration of polymers with different chemistry

Branched polyethylene (sample) relative to polystyrene (calibration standards)



Conventional calibration results

Sample	M_w	M_n	M_w/M_n
PE1476	171,454	20,314	8.440

Triple Detection method results

Sample	M_w	M_n	M_w/M_n
PE1476	86,508	19,139	4.520

Limitations of conventional calibration

- Every polymer has its own calibration line, which means that M_w values are only accurate for same polymer types
  - Only relative M_w obtained!
  - How close the true and relative M_w values are depends on how close the analyte and standards are in chemical composition and structure
- Any structural change, such as branching, will also affect the accuracy of this value
  - Gives relative M_w even further away from true M_w value!
  - Remember: compare apples with apples or at least a spherical fruit!
- Does not give structural information

Summary

Conventional calibration

- Simple technique to give whole polymer distribution
  - Need to take care with sample/solvent/column compatibility
  - Comparison of samples is easy
- Calibration is main difficulty
  - Data is therefore only relative
- Chromatography conditions need to be carefully controlled
  - Retention volume can be affected by change in conditions
- No structural information
  - Not useful for branched polymers

Software Exercise 3

Conventional Calibration on OmniSEC v5

Objectives

This exercise will instruct you on how to use the OmniSEC v5 software to:

- Estimate the molecular weight of two unknown sample using conventional calibration.

Learning Outcomes

Following this exercise, you will be able to use the OmniSEC v5 software to:

- Set baselines and limits.
- Process conventional calibration data using the OmniSEC software.
- Understand the factors affecting Conventional

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Go to exercise

The Triple Detection Method

- Read all the points
- Follow each steps
- Fill in the tables
- Answer questions