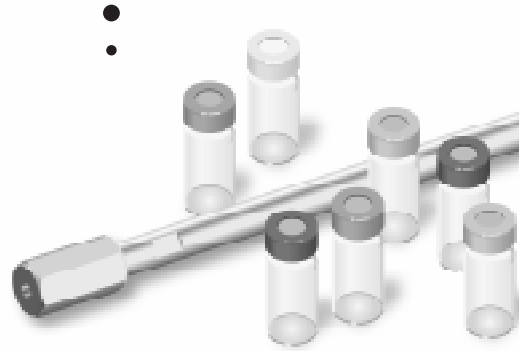

Getting Ready for GPC-SEC Analysis

Agilent Technologies

Agilent Part No. G2182-90100

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Agilent Technologies Deutschland GmbH
Chemical Analysis Group Europe
Hewlett-Packard-Strasse 8
76337 Waldbronn
Germany

Getting Ready for GPC-SEC Analysis

Start-up kits
for analyses with organic
and aqueous eluents

Organic GPC-SEC Start-up Kit **PN: 5064-8251**
Aqueous SEC Start-up Kit **PN: 5064-8252**

Upon receipt please verify that all kit contents listed on page 6 (Organic GPC-SEC Start-up Kit) and page 30 (Aqueous SEC Start-up Kit) are included. If any part is missing, contact your Agilent Technologies sales office.

Table of Contents:

C_6H_5

Part A

Getting Ready for GPC-SEC Analysis with Organic Eluents

Organic GPC-SEC Start-up Kit
PN: 5064-8251

I. Introduction

The kit "Getting Ready for GPC-SEC analysis with organic eluents" is developed to get you up and running quickly and easily your polymer samples. It contains one PLgel Mixed-C column (Agilent order number 79911GP-MXC), ready to use mixtures of calibration standards and this manual for the guided development of the data acquisition-, GPC data analysis- and reporting part of a broad polystyrene test sample in interactive and automatic mode. To ensure that the kit and the instruments are functioning properly, a detailed procedure and test chromatograms are given below. The influences of various parameters on the precision and accuracy of molecular weight data as well typical errors are discussed. An application chapter is encluded showing typical analyses with PLgel columns.
We assume that the user is familiar with the theory of GPC-SEC, the Agilent 1100 Series hardware, the ChemStation and the GPC data analysis software.

II. Kit contents and other supplies

Part	Quantity	Part Number
Column: PLgel Mixed-C, 300 x 7.5 mm, 5 µm	**1**	**79911GP-MXC**
Test certificate for the column	**1**	**n.a.**
PLgel User Guide	**1**	**n.a.**
Mixture of four EasyCal polystyrene standards (vial RED)*	**3**	**n.a.***
Mixture of four EasyCal polystyrene standards (vial BLUE)*	**3**	**n.a.***
Mixture of four EasyCal polystyrene standards (vial GREEN)*	**3**	**n.a.***
Characterization data for polystyrene standards	**1**	**n.a.**
Broad polystyrene test sample(vial Yellow)	**3**	**n.a.***
Instruction manual	**1**	**G2182-90100**

The polystyrene standards are available in a 10 x 3 vial quantity with part number 5064-8281.

The following parts/supplies should be ordered separately:

Part	Quantity	Part Number
Adapter (only needed with 1100 Series quaternary pump)	**1**	**0100-1847**
Tetrahydrofuran (THF) stabilized with BHT (Butylated Hydroxytoluene)	**2 L**	**To be ordered from local supplier**

(You may also use HPLC grade THF with the drawbacks of a higher baseline drift and a higher price. For applications with an additional UV detector stabilized THF can be used with wavelengths above approximately 250 µm.)

III. Instrumentation

a) Agilent Technologies 1100 Series GPC Analysis System

including:

- **Isocratic pump** (G1310A)
  If alternatively the binary or the quaternary pump is used **never** mix the eluent with the pump. In case of the binary pump strictly use 100% of channel A and 0% of channel B. The quaternary pump should be plumbed such that only one channel is used and the multi channel gradient valve is bypassed. This needs an additional adapter (P/N 0100-1847) to connect the solvent tube directly to the active inlet valve instead of the proportioning valve
- **Online degasser** (G1323A - strongly recommended)
- **Autosampler** (G1313A - strongly recommended)
- **Thermostatted column compartment** (G1316A - strongly recommended)
- **Refractive index detector** (RI – G1362A)
- **ChemStation** (G2170AA, revision A.06.04 or higher) with GPC data analysis software (G2182AA, revision A.01.03 or higher)

For obtaining reliable results the equipment should have passed an OQ/PV testing before.

b) Column

- **Agilent Technologies PLgel Mixed-C, 300 x 7.5 mm, 5 µm** (79911GP-MXC)

The PLgel Mixed-C column is packed with 5 µm particles of different pore sizes and thus enables the separation of polymers over a wide molecular weight range (from about 200 to 3 million). Such wide ranges require with traditional single pore size columns sets of several columns, typically between 2 to 3. On the other hand a 30 cm long mixed gel column will never have the same plate numbers as a complete column set. Therefore a typical application for this column is a fast, screening type analysis which can be afterwards optimized. This optimization is achieved either through the addition of further mixed gel or dedicated single pore size columns (refer to chapter X. Applications).

IV. Preparation

It is time saving if the chapters IV. Preparation and V. Instrument Parameters are performed in the afternoon of the first day and the other chapters on the following day. This gives the refractive index detector and the column sufficient time for conditioning during the night.

1. Add 1.0 mL THF to one vial of each of the standard mixtures (RED, BLUE and GREEN) and one broad sample vial (YELLOW) and allow dissolving for 60 minutes. Shake the vials every ten minutes. After addition of the solvent the vials should be used within two days.

2. Switch on all modules of the GPC system

3. Make sure that the system is free of salts or solvents immiscible with THF. Otherwise flush the system thoroughly with water or an intermediate solvent

4. Fill 1 liter of stabilized THF into the solvent bottle of the 1100 Series isocratic pump (for the binary and the quaternary pump refer to chapter Instrumentation)

5. Open the purge valve of the pump and purge the channel in use at 5 mL/min for at least 10 minutes

6. Close the purge valve and flush the pump, autosampler and column thermostat at 1 mL/min for at least 5 minutes without column.

V. Instrument parameters

1. Analytical conditions:
If the GPC data analysis software revision is A.02.01 or higher you can load the following methods from your hard disc using the below method names and the path C:\hpchem\X\Methods (where X is the instrument number):

- OK_STAN.M – for analysis of the polystyrene standards
- OK_SAM.M – for analysis of the broad polystyrene test sample

The two methods differ in the integration parameters and in the "Run Time Checklist". For the narrow standards and the broad polystyrene sample different parameters are needed because of different peak shape. They may need special adaptation to the requirements of your peaks determined by the GPC system and column performance (refer to chapter IX, Automatic calculation of molecular weight data).
The analysis of polymer standards is a conventional HPLC run and does not require the activation of the GPC data analysis software in the "Run Time Checklist".

If your GPC data analysis software has a revision number lower than A.02.01 key in the following parameters and save them afterwards using the above path and file names:

		Remarks
Pump:		
Eluent channel:	100%A	***strictly isocratic, no premixing!*** *Refer to chapter III. Instrumentation*
Flow rate:	1.0 mL/min	
Stop time:	12 min	
Max. pressure limit:	150 bar	
Min. pressure limit:	5 bar	
Autosampler:		
Injection volume:	50 µL	*standards and test sample*
Column thermostat:		
Temperature:	25 °C	*left and right side*
Refractive index detector:		
Optical unit temperature:	25 °C	
Polarity:	Positive	
Automatic recycling after analysis:	OFF	
Peak width:	0.1 min	

Use the enhanced integrator (both standard and test sample analysis).

Integrator settings for polystyrene standards(method AK_STAN.M):

		Remarks
Initial Slope Sensitivity:	0.500	
Initial Peak width:	0.500	
Initial Area Reject:	10000	
Initial Height Reject:	1000	
Initial Shoulders	OFF	
Time 0.000	Negative Peaks ON	*Negative peaks are integrated well*
Time 0.00	Integrator OFF	
Time 3.5	Integrator ON	*No peak elution possible before 3.5 minutes*

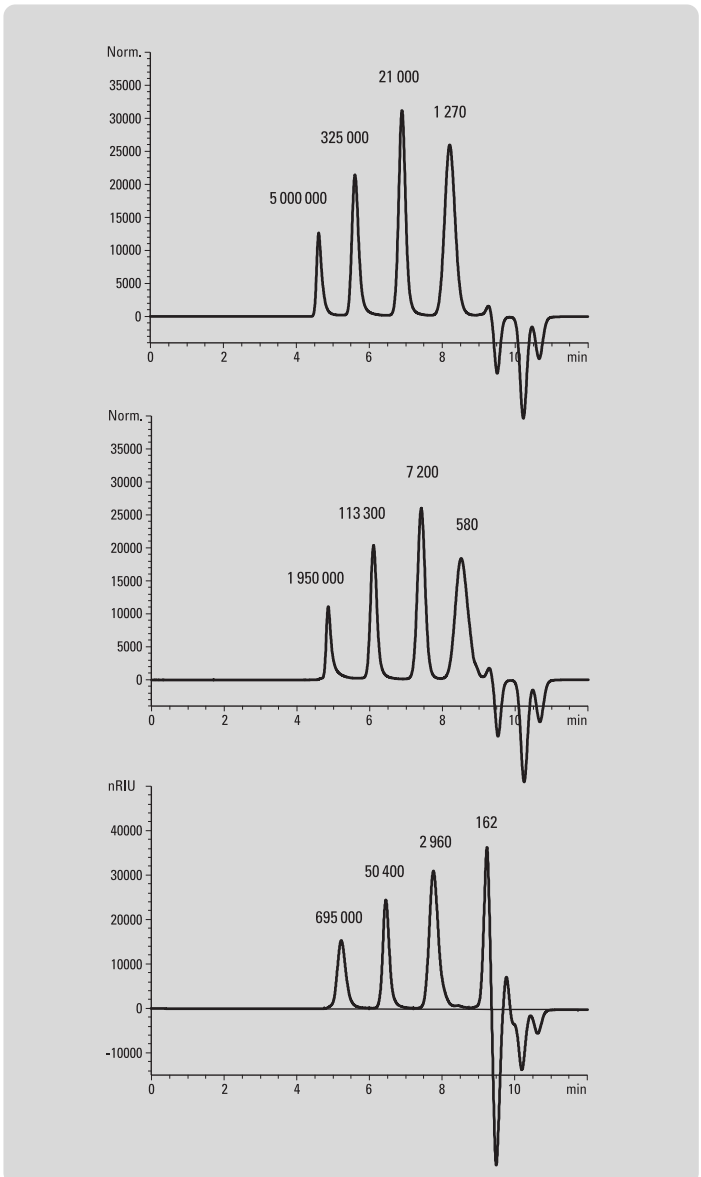
Integrator settings for broad polystyrene test vial (method OK_SAM.M)
(will need special adaptation to peak shape and retention times with your system):

		Remarks
Initial Slope Sensitivity:	1.00	*Adapted to broad polymer peak*
Initial Peak width:	1.00	*Adapted to broad polymer peak*
Initial Area Reject:	10000	
Initial Height Reject:	1000	
Initial Shoulders	OFF	
Time 0.000	Negative Peaks ON	*Negative peaks are integrated well*
Time 0.000	Integration OFF	*No elution of peaks possible before volume of total penetration*
Time 4.00	Integration ON	
Time 4.010	Baseline NOW	*Forces integrator to reset baseline*

2. Change the flow rate to 0.5 mL/min
3. Install the column in the thermostatted column compartment and connect the inlet of the column to the autosampler and the outlet to the refractive index detector. Pump eluent at 0.5 mL/min to waste for 30 minutes to replace the shipping solvent (toluene) with the eluent (THF). During this time purge the reference cell of the RI detector.
4. Increase the flow rate to 1 mL/min and pump for at least another 2 hours, at least until the detector baseline is stable, no drift and noise can be seen at a full scale setting of 5000 nRIU
(detector noise: $\leq \pm 1 \times 10^{-8}$ RIU,
detector drift: $\leq 3 \times 10^{-6}$ RIU/h,
wander: $\leq 3 \times 10^{-7}$ RIU). For the automated calculation of these detector parameters refer to the Verification (OQ/PV) part of your ChemStation.
5. For best precision and accuracy it is recommended to perform this conditioning over night.

VI. Data acquisition

1. Set up a sequence of two 50 µl injections of each standard (method AK_STAN.M) and the broad test vial (method OK_SAM.M) in the order of PS vial RED, PS vial BLUE, PS vial GREEN and broad PS vial YELLOW.
2. Check for baseline stability and start the sequence
3. Typical chromatograms for the injections with HPLC grade THF as mobile phase and as sample solvent are shown below.



Figures 1 – 3:
Typical chromatograms of calibration mixtures PS vial RED, PS Vial BLUE and PS vial GREEN

The first 4 peaks of the standard chromatograms are the narrow polystyrene standards with decreasing molecular weight. After the last standard peak elute monomer peaks due to solvent impurities. If you use stabilized THF you will see a large negative peak of the stabilizer. The other negative peaks are mainly caused by pressure drops during the injection cycle.
These monomer and pressure drop peaks can vary from instrument to instrument and with mobile phase and sample solvent.

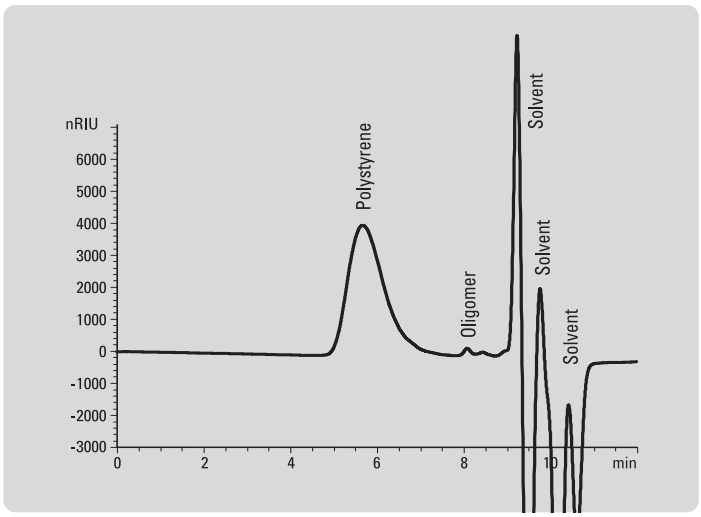


Figure 4:
Typical chromatogram of the polystyrene test sample (YELLOW)

VII. Calibration of the GPC system

Inspect the chromatograms carefully for repeatability of peak shapes and retention times to avoid stray points. With a good performing GPC system the retention times of two consecutive injections of the same polymer should in a maximum differ in the positions two and/or three behind the point. Consecutive injections from the same vial should look like a single chromatograms when overlaid. This should be the case for the positive peaks, the negative peaks may have a varying height caused by the pressure drop during the injection cycle. For a typical example refer to figure 5.

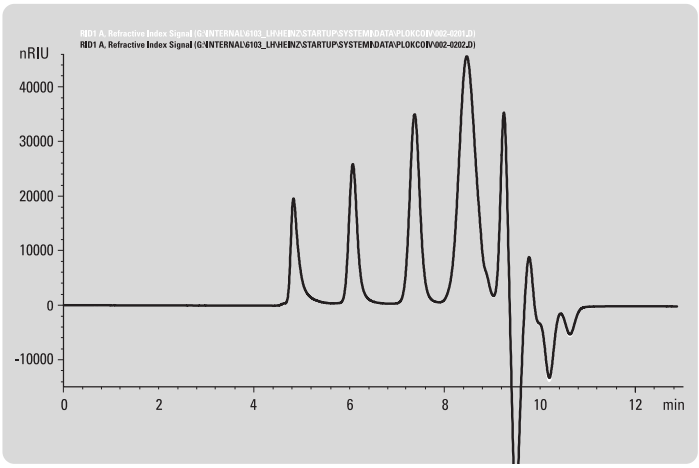


Figure 5:
Typical overlay of two consecutive calibration runs from vial BLUE

If the standard and the test sample chromatograms fulfill these conditions use them for calibration and evaluation, if not refer to chapter Troubleshooting. Because of this good precision of the retention times use for the present calibration only the second run of each vial. For a "real live" calibration and application you may use all data points.

1. In ChemStation Data Analysis load OK_STAN.M

2. In the [GPC] menu bar select [Activate GPC]

3. In [GPC Settings ...] inspect whether the GPC settings reflect your hardware configuration (e.g. detector configuration). Make sure that the default.cal calibration file is loaded and

that the “Report Settings” section of the GPC-Settings dialog are set to “Interactive Screen Review”. Press the OK button when finished to return to the Data Analysis view of the ChemStation.

4. Load the second data file of polystyrene standard vial RED and transfer it to the GPC data analysis software by pressing “Calculate GPC Results” of the GPC menu.

5. Highlight the Raw Data window (R) and open the sample editor by selecting [Sample] from the [Editor] menu. Enter the molar masses Mp of the 4 narrow standards of polystyrene standard vial RED with decreasing molar mass as they are displayed in the Certificate of Analysis. All the other inputs are not essential for the narrow standard calibration. Press the OK button when finished. Select [Window | Calibration] from the GPC data analysis menu and create an empty calibration file by clicking on [File | New]. The calibration window will change background color.

6. Highlight the Elugram window and move the cursor below the (first) peak and below the x-axis. Click on the right mouse button and select [Find Maximum] from the pop-up command box. There might be a difference in the retention times calculated by the ChemStation data analysis and the GPC data analysis part due to different algorithm used. In the Add to calibration dialog box select the proper molar mass of the calibration standard from the list by clicking on the correct radio button. Then add this calibration point to the calibration table by pressing the “add to calibration” button. Continue this for all standards in this chromatogram.

7. Then load the data files of polystyrene standard vials BLUE and GREEN from the Data Analysis and process them in the same way as described in steps 4 – 6 until you have added all calibration standards to the calibration table.

8. When you have completed the calibration table, activate the Calibration window in the GPC ADD-ON window and choose a regression model, e.g. Polynom 3, from the “Fit” drop-down selection list in order to create a calibration curve.

9. Try all possible regression models and optimize with the deviation data displayed in the calibration table. The fit quality should in addition always be controlled by the signs of the differences between the original calibration points and the values calculated by regression, using the absolute M-values and not their logarithms. The signs of these residues must be randomly distributed. The fit is insufficient if this is not the case [(1)].

10. You can check quickly the quality of the regression with the deviation plot to be selected from the "Compare" drop-down selection list.
Figure 6 shows a typical calibration curve with a 3rd order polynom fit. The points showing deviation are mostly located below the calibration curve indicating an insufficient fit.
Figure 7 shows the same calibration data with a 7th order fit. This fit is superior as shown by the random distribution of the deviation points and the lower deviations.

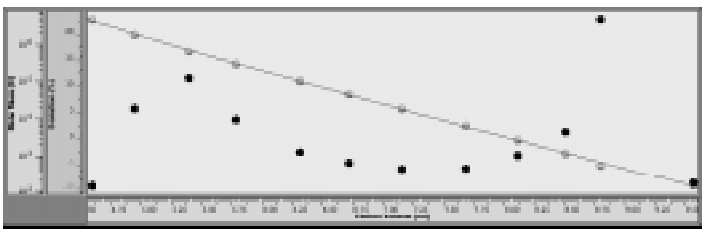


Figure 6:
Calibration curve for polystyrene standards obtained with an insufficient 3rd order fit (deviation points not randomly distributed)

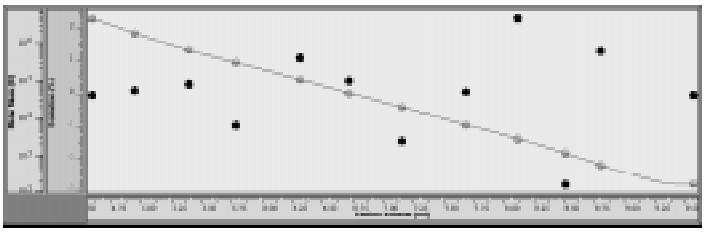


Figure 7:
Calibration curve for polystyrene standards obtained with a 7th order fit (deviation points randomly distributed)

To rely only on the above requirements can easily create errors. For example, for 6 calibration points it is always possible to fit a polynomonal of the fifth degree such that the calculated curve runs through all

calibration points. However you usually receive swinging curves, i.e. curves which have partially increasing slope, which is physically meaningless. The following requirements should be fulfilled in addition:

- The slope of the curve should be physically meaningful. You can view the derivative of the calibration curve in the respective column of the editor section and as graphical information by selection from the Compare list
- The slope of the calibration curve should be highly negative for small and large elution volumes while there should be a broad region with relatively constant value within.

11. Choose [file | print] to make a printout of the calibration table and graph.

12. Save the calibration file using the [Save As] dialog from the File menu and give a descriptive name for this calibration file, e.g. OK_Calib.cal.

VIII. Interactive calculation of molecular weight data

1. In ChemStation Data Analysis load the method OK_SAM.M.
2. Load a data file obtained for the broad polystyrene test sample.
3. Select [GPC], then [GPC Settings] and load the calibration file OK_Calib.cal you have saved in the end of chapter calibration
4. Press OK and then Save method OK_SAM.M, the calibration file OK_Calib.cal is now saved with the method OK_SAM.M
5. Next select the [GPC] menu and then [Calculate GPC Results]
6. The file is transferred to the GPC data analysis software which displays the GPC-Addon top-level window with the broad test sample as example

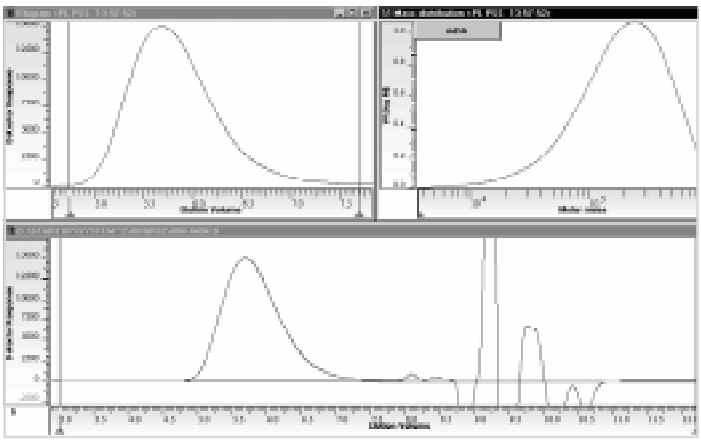


Fig. 8:
Top-level window of the GPC data analysis software for broad PS test sample

a) Defining the baseline

7. In the raw data-(R) part define next the baseline by moving the red triangles to the correct positions. Ideally the baseline should be taken as a straight line between the elution prior to the size exclusion limit, about 4 mL, and that after the last impurity peak, i.e. areas in which no elution can take place in an ideal GPC separation[1,3], refer to figure 9. For setting the baseline correctly make sure by zooming in to about 10% of total peak height that there is

   - no peak elution at the position of the red triangles
   - no cut off of the polymer peak

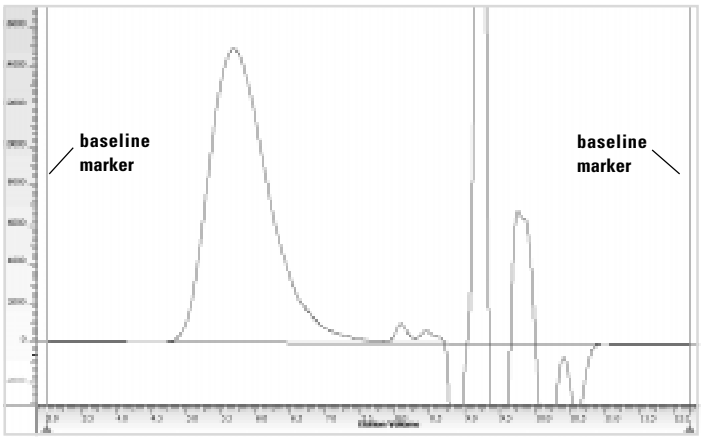


Figure 9:
Raw data window for broad PS test sample with baseline markers set prior to volume of total exclusion and after last impurity peak

If this drawing of the baseline does not work properly – e.g. peaks from previous analysis elute prior to the size exclusion limit or the baseline is not sufficiently stable after the last impurity peak – set the baseline markers exactly in the position where the polymer peak starts and ends. For better seeing this you have to zoom in.

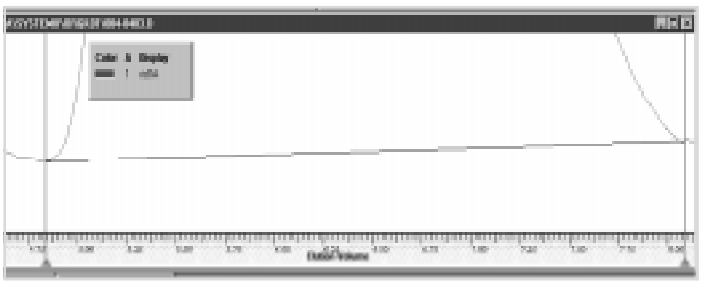


Fig. 10:
Raw data window for broad PS test sample with baseline markers set at peak start and end

Baseline setting as shown in figure 10 has the drawback of less flexibility for setting the integration limits in the Elugram (E) window (refer to below) but is often a more practical approach.

b) Defining the integration limits

In the Elugram window the baseline corrected raw data are presented.
In the Elugram window the integration limits for calculation of the molecular weight distribution and the molecular weight averages have to be set with the red markers. Position them exactly before the start and exactly at the end of the peak. You will have to zoom in for better seeing this. This step is typically not needed if you set the baseline as shown in figure 10.

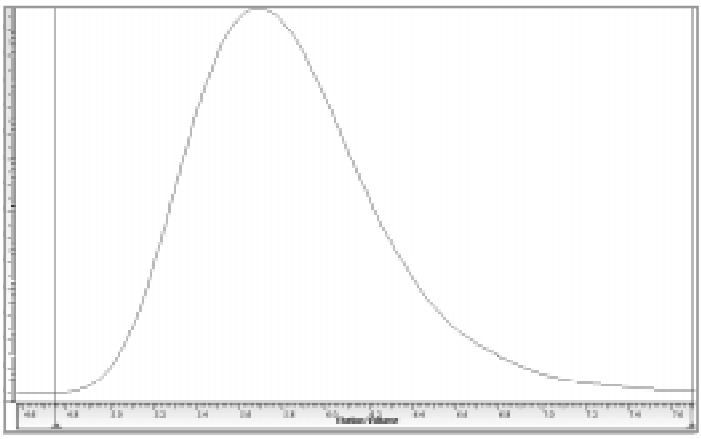


Fig. 11:
Elugram window showing correct position of integration limits

During the optimization of baseline and integration limits study the drastic influence of the position of the triangles on the molecular weight results. You can change especially the M_n-value drastically with the set points. This strong influence of the integration limits and the baseline setpoints on the molecular weight data is one of the reasons for the limited precision and reproducibility of the molecular weight averages.

A typical value for the M_w-value of the broad polystyrene sample under the chosen conditions is:

Molecular weight	Typical GPC value	Limits (± 20%)
M_w	265000	212000 - 318000

The M_w-data should be in a range of ± 20% within the typical value. Try to come closest to the reference value If you cannot succeed refer to Part C Appendices, chapter I. Troubleshooting.

The lab to lab reproducibility of molecular weight data depends on several hardware and software parameters as the table below shows:

Hardware Parameters	Software Parameters
• Column performance • Flow precision • Temperature precision • Baseline stability • Injection volume precision • Quality of the standards	• Accuracy of calculation procedures • Quality of baseline setting • Quality of setting the calculation start and end marks • Quality of calibration curve • Number of data points • Possibility to use an internal standard correction for flow rate changes

Because of the many influencing parameters lab to lab reproducibility is often poor. R. Bruessau reports [(1)] of an European round robin test which resulted in differences from lab to lab for M_n of ± 16 % and for M_w of ± 9%. Similar data were reported from a Japanese round robin test[(2)]. In both round robbin studies only experienced GPC laboratories participated!

8. Highlight the [Mass Distribution(M)] window, then select the [Raw Data] menu and press [Print]. You will get a single page report printed consisting of the mass distribution, the main method parameters and in the bottom the molecular weight data.

9. The molar mass distribution should look like the one shown in figure 12.

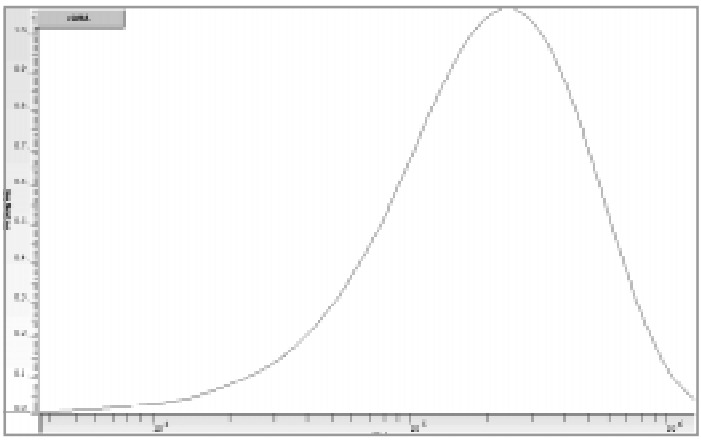


Fig. 12:
Typical molecular weight distribution of the broad polystyrene sample

10. Determine the molecular data in the same way for the second data file obtained with the broad polystyrene vial (YELLOW).

IX. Automatic calculation of molecular weight data

This chapter explains how to set up the system for completely automatic analyses consisting of data acquisition, GPC data analysis and reporting based on the above developed method. An Agilent 1100 Series GPC analysis system with degasser, pump, autosampler, thermostatted column compartment and refractive index detector is required.

a) Preparation and start

1. Make sure that the system is still running using the above conditions and that method OK_SAM.M is loaded. In the [GPC Settings] window the calibration file OK_CALIB.CAL is activated.

2. Load the last data file obtained for the broad polystyrene test sample.

3. The integration parameters in OK_SAM.M need to be optimized for the peak shape obtained for the broad polystyrene with your system. The GPC data analysis part of the ChemStation calculates in a sequence the molecular weight data between the start mark of the 1st integrated and the end mark of the last integrated peak. It is therefore extremely important that

- Only the polymer peak is integrated (refer to figure 4)
- The start and end integration marks of the polymer peak are placed where the peak starts and ends (zoom in several times for better seeing this, refer also to chapter VIII and figure 10)
- For optimizing the start and end integration marks use:

	Remarks
• **Slope sensitivity**	*Big impact on start and end mark (Use values as 0.01 or 1000 for studying the influence)*
• **Peak width**	*Big impact on start and end mark (Use values as 0.01 or 10 for studying the influence)*
• **Negative peaks On**	*Negative peaks are integrated well and baseline is not drawn to minimum of negative peaks*
• **Integration On/Off**	*For suppression of peaks of no interest*
• **Baseline Now**	*Forces integrator to reset baseline*

For more information on the integration parameters and events refer to the ChemStation help text.

Change the above parameters until the start and end marks are optimized. Correct drawing of the baseline itself within the standard Data Analysis is not important. The GPC data analysis itself draws the baseline arbitrarily from integration start to end mark.

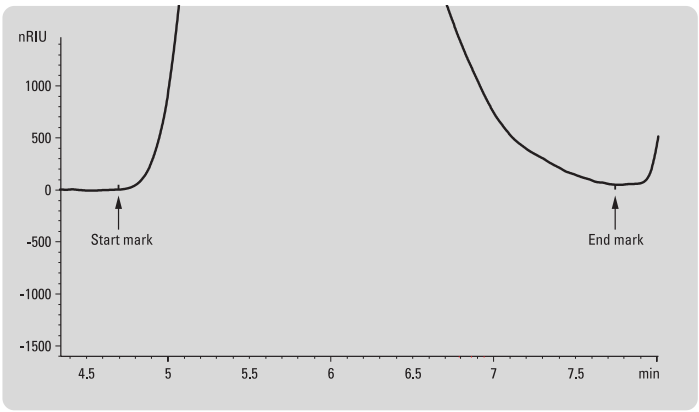


Fig. 13:
Example for broad test sample with correctly set integration marks

4. Go into the [GPC Settings…] of the [GPCmenu] and
   - Change the Report Settings from "Interactive Screen Review" to "Print Results"
   - Configure a report consisting of e.g. "Graphical MWD", a "Method"- and a "Results" part
5. Save the method again and set up a sequence consisting of 6 injections of the broad polystyrene test sample
6. Check for baseline stability and start the sequence

b) Evaluation

1. You should have received the following printouts for each run automatically
   - One standard HPLC report
   - One single page GPC report consisting of one "Graphical MWD", one "Method"- and one "Results" part
2. In the ChemStation Data analyis overlay the chromatograms of the six analyses

3. The overlay should look with a well-conditioned column and a well-maintained GPC system visually like a single chromatogram at least in the polymer region. If this is not the case the precision of the molecular weight data may be negatively affected.

The negative peaks may have a varying height caused by the pressure drop during the injection cycle.

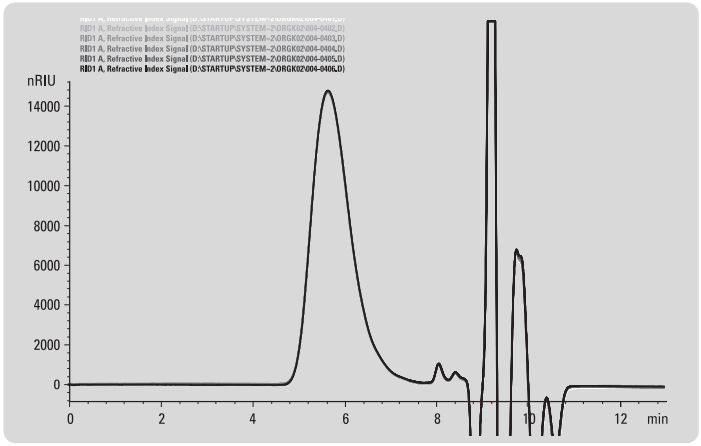


Fig. 14:
Overlay and zoom in into 6 automatic runs of broad PS test sample

3. Calculate the Standard Deviation (SD) and Relative Standard Deviation (%RSD) for the 6 obtained M_n- and M_w-values using the following formulas on your pocket calculator:

$$\text{Standard Deviation} = \sqrt{\frac{n}{n-1}\sum(x-\bar{x})^2}$$

$$\%\ \text{RSD} = \frac{SD}{\bar{x}} \cdot 100$$

n = number of runs
x = actual value of M_n or M_w
$\bar{x}$ = average Value of M_n or M_w

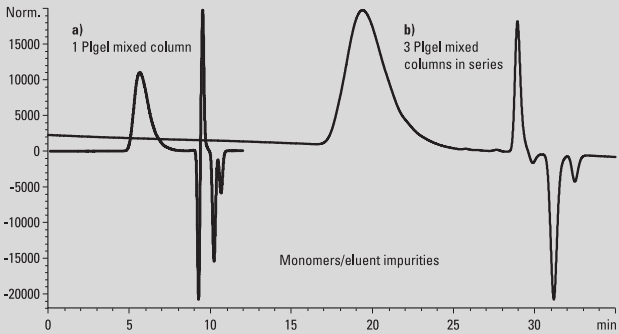
4. The relative standard deviations should be
- For M_n < 10 %
- For M_w < 7 %

If this is not the case refer to part C chapter I. Troubleshooting. For more information on precision of molecular weight data achievable with optimized, state of the art equipment refer to literature 5)

X. Applications

a) Example of method development

The chromatogram shows the analyses of a broad technical polystyrene: part a) with one PLgel Mixed-C column and part b) with a set of three PLgel Mixed-C columns. Due to the better separation of the column set and the reduced influence of band broadening, the calculated molecular weight averages are in the later case closer to the values determined with reference methods, e.g. light scattering (refer to table).



Columns:	a) 1 x PLgel Mixed-C, 7.5 x 300 mm, 5 µm (Agilent Part No. 79911GP-MXC)
	b) 3 x PLgel Mixed-C, 7.5 x 300 mm, 5 µm in series
Mobile phase:	Tetrahydrofuran (THF)
Column temp.:	25 °C
Flow rate:	1 mL/min
Detector:	Refractive Index Detector
Calibrant:	Polystyrene EasyCal standards in vials for calibration

Fig. 15:
Broad polystyrene with 1 and 3 PLgel Mixed columns

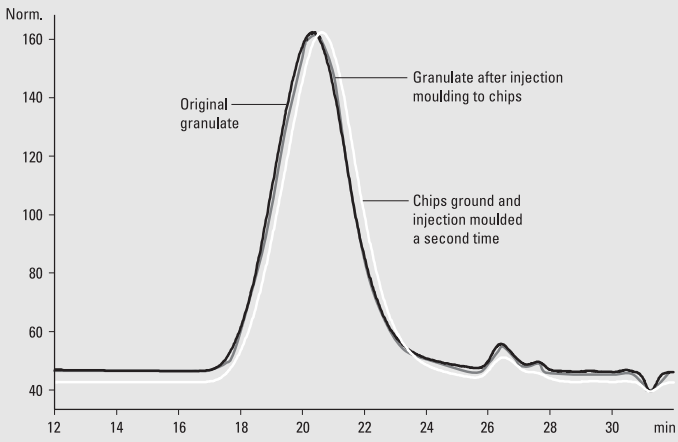
Reference Data	**M_n* 86000**	**M_w** 246000**	**D 2.86**
Difference [%] (1 x PLgel Mixed)	9	15	27
Difference [%] (3 x PLgel Mixed)	3.1	4.8	2.0

* measured by GPC
** measured by light scattering

b) Example of process control

This figure shows an overlay of 3 chromatograms of another technical polystyrene – the original granulate and before and after injection molding. After the first injection molding, there is almost no change in the chromatogram and therefore the molecular weight distribution. After grinding the chips, and injection molding a second time there is a significant change which will have an effect on the properties. The visual information is supported by the number average molecular weight, M_n, as calculated by the ChemStation GPC data analysis software:

M_n (original granulate): 59000
M_n (after 2nd process): 55000



Columns:	3 x PLgel Mixed-B columns, 7.5 x 300 mm, 5 µm (Agilent Part No. 79911GP-MXB)
Mobile Phases:	Tetrahydrofuran (THF)
Flow rate:	1 mL/min
Oven temp:	20 °C
Injection Volume:	10 µL
Detector:	Refractive Index Detector
Sample:	dissolved in 1 mL THF
Calibrant:	EasyCal polystyrene standards in vials were used for narrow standard calibration

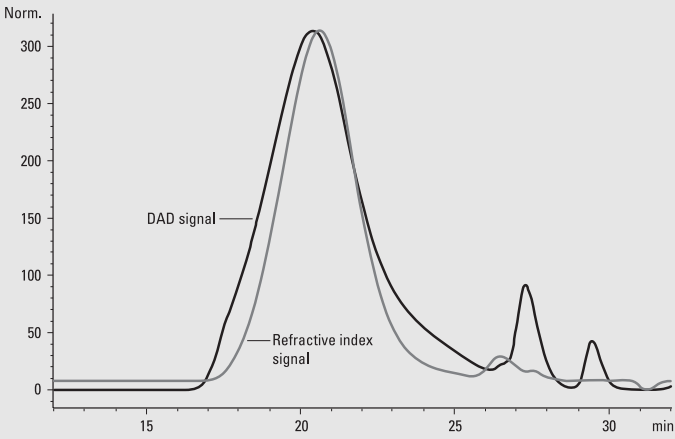
Fig. 16.
Example of process control

c) Example of quality control

About 1.5 million tons of PMMA are produced on a worldwide basis for applications as

- plastic glass, e.g. for roofs,
- safety glasses,
- glasses for cars and dishes.

The quality of the polymer strongly depends on the molecular weight and the molecular weight distribution. The figure shows the overlay of a Refractive Index and a UV detector signal of a PMMA with a weight average molecular weight (M_w) of 316000.



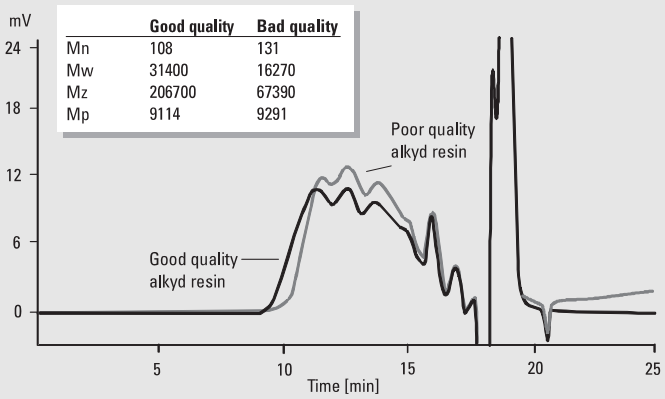
Columns:	**3 x PLgel Mixed-C in series, 7.5 x 300 mm, 5 µm (Agilent Part No. 79911GP-MXC)**
Mobile phase:	**Tetrahydrofuran (THF)**
Flow rate:	**1.0 mL/min**
Oven temp.:	**20 °C**
Injection volume:	**10 µL**
Detector:	**Refractive Index Detector and Diode Array Detector**
Sample:	**26 mg dissolved in 1 mL THF**
Calibrant:	**EasyCal Polystyrene standards in vials were used for narrow standard calibration**

Fig. 17:
PMMA polymer with M_w = 316000

d) Example of quality control

Alkyd resins are widely used for the production of paints. This example shows the quality control analysis of two resins used for high quality paints in the car industry.

The paint failed in the application when produced with the bad quality resin, while it had excellent adhesive properties when produced with the good quality resin.



	Good quality	Bad quality
Mn	108	131
Mw	31400	16270
Mz	206700	67390
Mp	9114	9291

Columns:	**PLgel 10 ^{2} Å, 7.5 x 300 mm, 5 µm (Agilent Part No. 79911GP-501) in series with PLgel 5 x 10 ^{2} Å, 7.5 x 300 mm, 5 µm (Agilent Part No. 79911GP-502) and PLgel 10 ^{4} Å, 7.5 x 300 mm, 5 µm (Agilent Part No. 7991GP-504)**
Mobile phase:	**Tetrahydrofuran (THF)**
Flow rate:	**1.5 mL/min**
Oven temp.:	**20 °C**
Injection volume:	**10 µL**
Detector:	**Refractive Index Detector**

Fig. 18:
GPC-SEC analysis in quality control:
Resin quality in paint

For more application examples refer to "Polymer and Hydrocarbon Processing Solutions Guide", Agilent publication number 12-5968-7020E.

C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
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C_6H_5
C_6H_5
C_6H_5

Part B

Getting Ready for SEC Analysis with Aqueous Eluents

Aqueous SEC Start-up Kit
PN: 5064-8252

I. Introduction

The kit “Getting Ready for GPC-SEC with Aqueous Eluents” is developed to get you up and running quickly and easily your polymer samples. It contains one PL aquagel-OH Mixed column (Agilent order number 79911GF-MXA, ready to use mixtures of calibration standards and this manual for the guided development of the data acquisition-, GPC data analysis and reporting part of a broad dextran test sample in interactive and automatic mode. To ensure that the kit and the instruments are functioning properly, a detailed procedure and test chromatograms are given below.

The influences of various parameters on the precision and accuracy of molecular weight data as well typical errors are discussed. An application chapter is encluded showing typical analyses with PL aquagel columns.

We assume that the user is familiar with the theory of GPC/SEC, the 1100 Series hardware, the ChemStation and the GPC data analysis software.

II. Kit contents and other supplies:

Part	Quantity	Part Number
Column: PL aquagel-OH Mixed, 8 mm, 7.5 x 300 mm	1	79911GF-MXA
Test certificate for the column	1	n.a.*
PL aquagel-OH user guide	1	n.a.*
Mixture of four EasyCal polyethyleneoxide standards PEO/PEG vial RED*	3	n.a.*
Mixture of four EasyCal polyethyleneoxide standards PEO/PEG vial BLUE*	3	n.a.*
Mixture of four EasyCal polyethyleneoxide standards PEO/PEG vial GREEN*	3	n.a.*
Characterization data for PEO/PEG standards	1	n.a.
Broad dextran test sample	3	n.a.
Instruction manual	1	G2182-90100

The polyethyleneoxide/polyethyleneglycol standards are available in a 10 x 3 vial quantity with part number 5064-8280.

The following parts/supplies should be ordered separately:

Part	Quantity	Part Number
Adapter (only needed with 1100 Series quaternary pump)	1 L	0100-1847
Water (HPLC grade)	2 L	To be ordered from local supplier

III. Instrumentation

a) Agilent Technologies 1100 Series GPC analysis system

including:

- **Isocratic pump** (G1310A)
  If alternatively the binary or the quaternary pump is used **never** mix the eluent with the pump. In case of the binary pump strictly use 100% of channel A and 0% of channel B. The quaternary pump should be plumbed such that only one channel is used and the multi channel gradient valve is bypassed. This needs an additional adapter (P/N 0100-1847) to connect the solvent tube directly to the active inlet valve instead of the proportioning valve
- **Online degasser** (G1323A – strongly recommended)
- **Autosampler** (G1313A – strongly recommended)
- **Thermostatted column compartment** (G1316A – strongly recommended)
- **Refractive index detector** (RI – G1362A)
- **ChemStation** (G2170AA, revision A.06.04 or higher) with GPC data analysis software (G2182AA, revision A.01.03 or higher)

For obtaining reliable results the equipment should have passed an OQ/PV testing before.

b) Column

- **Agilent Technologies PL aquagel-OH Mixed, 8 mm, 7.5 x 300 mm, (79911GF-MXA)**

The PL aquagel-OH Mixed is packed with 8 µm particles of different pore sizes and thus enables the separation of polymers over a wide molecular weight range (from about 100 to 10000000). Such wide ranges require with traditional single pore size columns sets of several columns, typically between 2 to 3. On the other hand a 30 cm long mixed gel column will never have the same plate numbers as a complete column set. Therefore a typical application for this column is a fast, screening type analysis which can be afterwards optimized. This optimization is achieved either through the addition of further mixed gel or dedicated single pore size columns (refer to chapter X. Applications).

IV. Preparation

It is time saving if the chapters IV. Preparation and V. Instrument Parameters are performed in the afternoon of the first day and the rest on the following day. This gives the refractive index detector and the column sufficient time for conditioning during the night.

1. Add 1.0 mL water to one vial of each of the standard mixtures (RED, BLUE and GREEN) and one of the broad sample vials (YELLOW) and allow dissolving for 60 minutes. Shake the vials every ten minutes. After addition of the solvent the vials should be used within two days.

2. Switch on all modules of the GPC system

3. Make sure that the system is free of solvents immiscible with water. Otherwise flush the system thoroughly with an intermediate solvent

4. Fill 1 liter of HPLC grade water into the solvent bottle of the 1100 Series isocratic pump (for the binary and the quaternary pump refer to chapter Instrumentation)

5. Open the purge valve of the pump and purge the channel in use at 5 mL/min for at least 10 minutes

6. Close the purge valve and flush the pump, autosampler and column thermostat at 1 mL/min for at least 5 minutes

V. Instrument parameters

1. Analysis conditions:
If the GPC data analysis software revision is A.02.01 or higher load the following methods from your harddisc using the following method names and the path C:\hpchem\X\Methods (where X is the instrument number):

- AK_STAN.M – for analysis of the PEO standards
- AK_SAM.M – for analysis of the broad dextran test sample

The two methods differ in the integration parameters and in the "Run Time Checklist". For the narrow standards and the broad dextran sample different integration parameters are needed because of too different peak shape. They may need adaptation to the requirements of your peaks determined by GPC system and column performance (refer to chapter IX, Automatic calculation of molecular weight data). The analysis of polymer does not require the activation of the GPC data analysis software in the "Run Time Checklist".

If your GPC data analysis software has a revision number < A.02.01 key in the following parameters and save them afterwards using the above path and file names:

		Remarks
Pump:		
Eluent channel:	100% A	***strictly isocratic, no premixing!*** *Refer to chapter III. Instrumental*
Flow rate:	1.0 mL/min	
Stop time:	12 min	
Max. pressure limit:	140 bar	
Min. pressure limit:	2 bar	
Autosampler:		
Injection volume:	50 µL	
Column thermostat:		
Temperature:	25 °C	*left and right side*
Refractive index detector:		
Optical unit temperature:	25 °C	
Polarity:	Positive	
Automatic recycling after analysis:	OFF	
Peak width:	0.1 min	

Use the enhanced integrator.

Integrator settings for PEO standards (method AK_STAN.M):

		Remarks
Initial Slope Sensitivity:	0.500	
Initial Peak width:	0.500	
Initial Area Reject:	10000	
Initial Height Reject:	1000	
Initial Shoulders:	OFF	
Time 0.000	Negative Peaks ON	*Negative peaks are integrated well*

Integrator settings for broad dextran test vial (method AK_SAM.M)
(may need special adaptation to peak shape and retention times with your system):

		Remarks
Initial Slope Sensitivity:	1.000	*Adapted to broad polymer peak*
Initial Peak width:	1.00	*Adapted to broad polymer peak*
Initial Area Reject:	10000	
Initial Height Reject:	1000	
Initial Shoulders	OFF	
Time 0.000	Negative Peaks ON	*Negative peaks are integrated well*
Time 0.000	Integration OFF	*No elution of peaks possible before volume of total exclusion*
Time 4.00	Integration ON	
Time 4.01	Baseline NOW	F*orces integrator to reset baseline, e.g. immediately before the peak*

2. Change the flow rate to 0.5 mL/min

3. Connect the inlet of the column to the autosampler and the outlet to the refractive index detector and pump eluent at 0.5 mL/min to waste for 30 minutes to replace the shipping solvent (water + 0.1% sodiumazide) with the eluent. During this time purge the reference cell of the RI detector.

4. Increase the flow rate to 1 mL/min and pump for another 30 minutes, at least until the detector baseline is stable, no drift and noise can be seen at a full scale setting of 5000 nRIU (detector noise: $\leq \pm 1 \times 10^{-8}$ RIU, detector drift: $\leq 3 \times 10^{-6}$ RIU/h, wander: $\leq 3 \times 10^{-7}$ RIU). For the automated calculation of these detector parameters refer to the Verification (OQ/PV) part of your ChemStation.

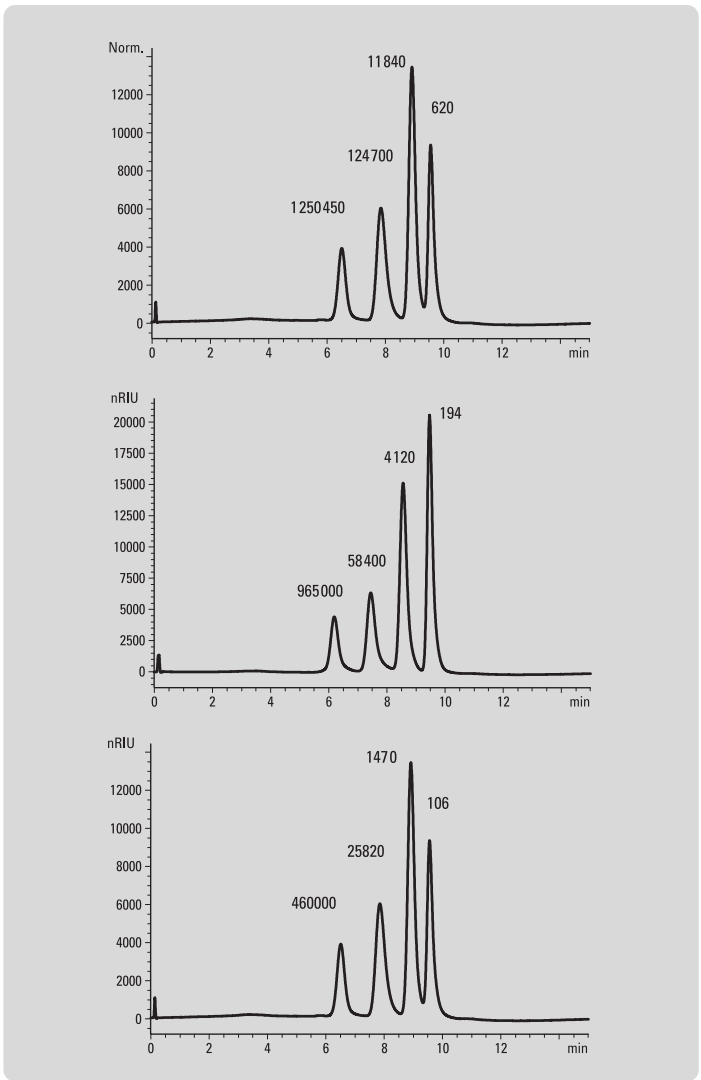
5. For best precision and accuracy it is recommended to perform this conditioning over night.

VI. Data acquisition

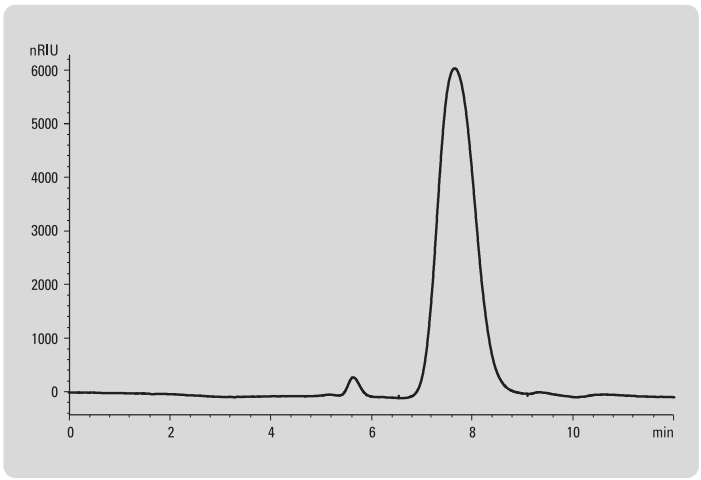
1. Set up a sequence of two 50 µL injections of each standard (method AK_STAN.M) and the broad test vial (method AK_SAM.M) in the order of vial RED, vial BLUE, vial GREEN and broad dextran vial YELLOW. The dextran sample inject 3 times and discard the first injection. Typically it takes one injection of the dextran until stable conditions for the column are obtained.

2. Check for baseline stability and start the sequence

3. Typical chromatograms for the injections are shown below.



Figures 1 – 3:
Typical chromatograms of calibration mixtures vial RED, vial BLUE and vial GREEN



Figures 4:
Typical chromatogram of the dextran test sample (YELLOW)

VII. Calibration of the GPC system

Inspect the chromatograms carefully for repeatability of peak shapes and retention times to avoid stray points. With a good performing GPC/SEC system the retention times of two consecutive injections of the same polymer should in a maximum differ in the positions two and/or three behind the point. Consecutive injections from the same vial should look like a single chromatograms when overlaid. For a good example refer to figure 5.

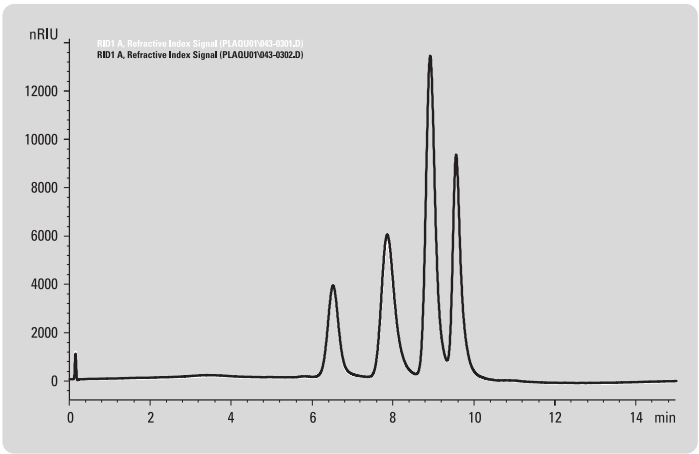


Figure 5:
Good overlay of two consecutive calibration runs from vial Green

If the standard and the test sample chromatograms fulfill these conditions use them for calibration and evaluation, if not refer to chapter Troubleshooting. Because of this good precision of the retention times use for the present calibration only the second run of each vial. If later on a higher precision is needed use all data points.

1. In Data Analysis load AK_STAN.M
2. In the [GPC] menu select [Activate GPC]
3. In [GPC Settings ...] inspect whether the GPC settings reflect your hardware configuration (e.g. detector configuration). Make sure that the default.cal calibration file is loaded and that the “Report Settings” section of the GPC-Settings dialog are set to “Interactive Screen Review”. Press the OK button when finished to return to the Data Analysis view of the ChemStation.

4. Load the second data file of PE0 standard vial RED and transfer it to the GPC data analysis software by pressing “Calculate GPC Results” of the GPC menu.

5. Highlight the Raw Data window (R) and open the sample editor by selecting [Sample] from the [Editor] menu. Enter the molar masses Mp of the 4 narrow standards of PE0 standard vial RED as they are displayed in the Certificate of Analysis. All the other inputs are not essential for the narrow standard calibration. Press the OK button when finished. Select [Window | Calibration] from the GPC data analysis menu and create an empty calibration file by clicking on [File | New]. The calibration window will change background color.

6. Highlight the Elugram window and move the cursor below the (first) peak and below the x-axis. Click on the right mouse button and select [Find Maximum] from the pop-up command box. There might be a difference in the retention times calculated by the ChemStation data analysis and the GPC data analysis part due to different algorithm used. In the Add to calibration dialog box select the proper molar mass of the calibration standard from the list by clicking on the correct radio button. Then add this calibration point to the calibration table by pressing the “add to calibration” button. Continue this for all standards in this chromatogram.

7. Then load the data files of PEO standard vials BLUE and GREEN from the Data Analysis and process them in the same way as described in steps 4 – 6 until you have added all calibration standards to the calibration table.

8. When you have completed the calibration table, activate the Calibration window in the GPC ADD-ON window and choose a regression model, e.g. Polynom 3, from the “Fit” drop-down selection list in order to create a calibration curve.

9. Try all possible regression models and optimize with the deviation data displayed in the calibration table. The fit quality should in addition always be controlled by the signs of the differences between the original calibration points and the values calculated by regression, using the absolute M-values and not their

logarithms. The signs of these residues must be randomly distributed. The fit is insufficient if this is not the case [1].

10. You can check quickly the quality of the regression with the deviation plot to be selected from the "Compare" drop-down selection list.
Figure 6 shows a typical calibration curve with a 3rd order polynom fit.
Figure 7 shows the same calibration data with a 7th order fit. This fit is superior as shown by the lower deviation values, in addition the deviation points are randomly distributed(figure 8).

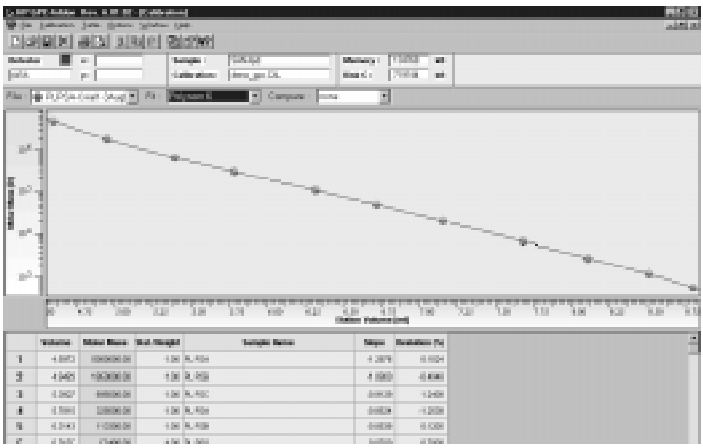


Figure 6:
Calibration curve for PEO standards obtained with 3rd order fit

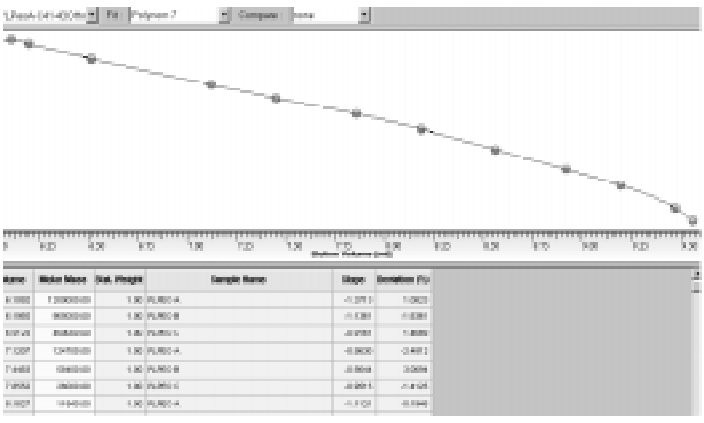


Figure 7:
Calibration curve for PEO standards obtained with a 7th order fit

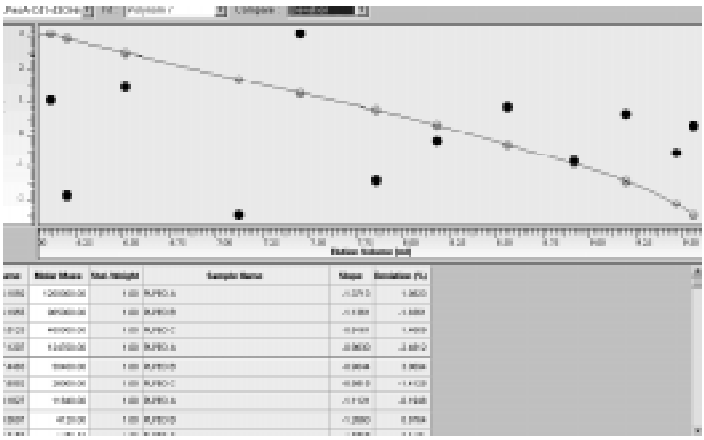


Figure 8:
Calibration curve ● for PEO standards obtained with a 7th order fit and randomly distributed deviation points ●

To rely only on the above requirements can easily create errors. For example, for 6 calibration points it is always possible to fit a polynominal of the fifth degree such that the calculated curve runs through all calibration points. However you usually receive swinging curves, i.e. curves which have partially increasing slope, which is physically meaningless. The following requirements should be fulfilled in addition:

- The slope of the curve should be physically meaningful. You can view the derivative of the calibration curve in the respective column of the editor section and as graphical information by selection from the Compare list
- The slope of the calibration curve should be highly negative for small and large elution volumes while there should be a broad region with relatively constant value within.

11. Choose [file | print] to make a printout of the calibration table and graph.

12. Save the calibration file using the [Save As] dialog from the File menu and give a descriptive name for this calibration file, e.g. AK_Calib.cal.

VIII. Interactive calculation of molecular weight data

1. In ChemStation Data Analysis load the method AK_SAM.M
2. Load a data file obtained for the broad dextran test sample.
3. Select [GPC], then [GPC Settings] and load the calibration file AK_Calib.cal you have saved in the end of chapter calibration
4. Press OK and then Save method AK_SAM.M, the calibration file AK_Calib.cal is now saved with the method AK_SAM.M
5. Next select the [GPC] menu and then [Calculate GPC Results]

   The file is transferred to the GPC data analysis software which displays the GPC-Addon top-level window

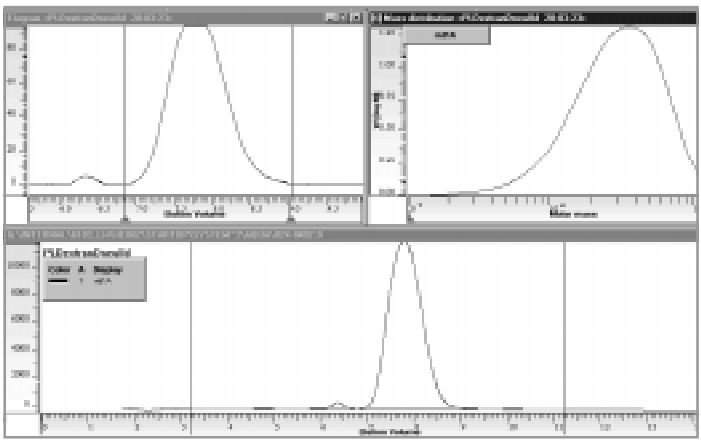


Figure 9:
Top-level window of the Agilent GPC data analysis software for broad dextran test sample

a) Defining the baseline

In the raw data-(R) part define next the baseline by moving the red triangles to the correct positions. Ideally the baseline should be taken as a straight line between the elution prior to the size exclusion limit, about 5 mL, and that after the last impurity peak, i.e. areas in which no elution can take place in an ideal GPC-SEC separation[1,3], refer to figure 9. For setting the baseline correctly make sure by zooming that there is

- no peak elution at the position of the red triangles
- no cut off of the polymer peak

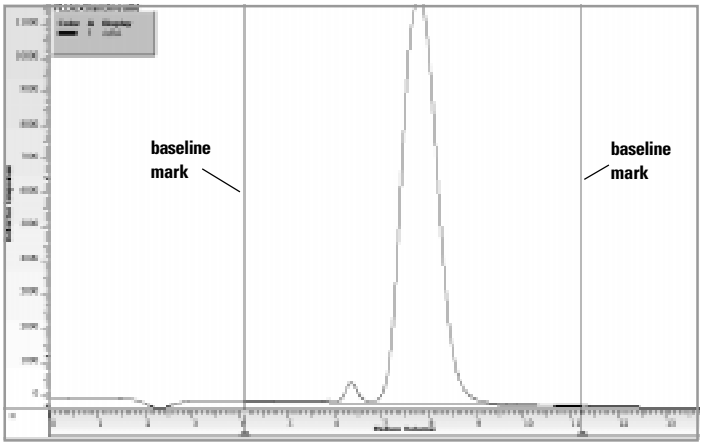


Figure 10:
Raw data window for broad dextran test sample with baseline markers set prior to size exclusion and last impurity peak

If this drawing of the baseline does not work properly – e.g. peaks from previous analysis elute prior to the size exclusion limit, or the baseline is not sufficiently stable after the last impurity peak – you may set the baseline markers exactly in the position where the polymer peak starts and ends. For better seeing this you have to zoom in.

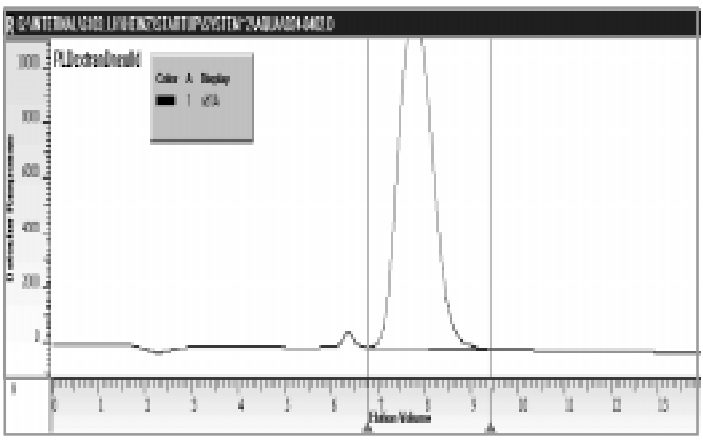


Fig. 11:
Raw data window for broad dextran test sample with baseline markers set at peak start and end

Baseline setting as shown in figure 11 has the drawback of less flexibility for setting the integration limits in the Elugram (E) window (refer to part b) but is often the more practical approach.

b) Defining the integration limits

In the Elugram window the baseline corrected raw data are presented.
In the Elugram window the integration limits for calculation of the molecular weight distribution and the molecular weight averages have to be set with the red markers. Position them exactly before the start and exactly at the end of the peak. You will have to zoom in for better seeing this. This step is typically not needed if you set the baseline as shown in figure 11.

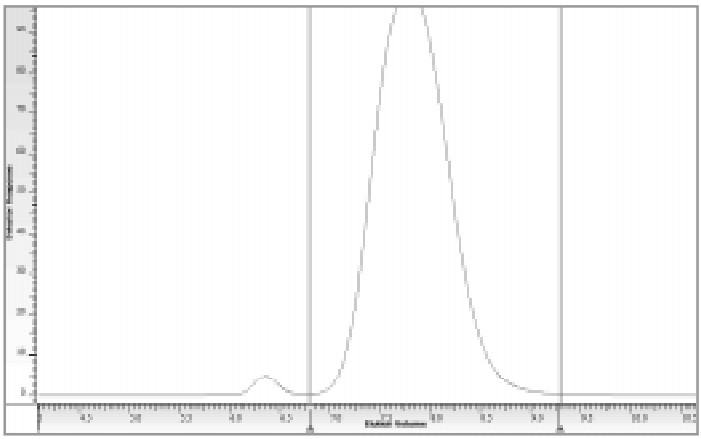


Fig. 12:
Elugram window showing correct position of integration limits

During the optimization of baseline and integration limits study the drastic influence of the position of the triangles on the molecular weight results. You can change especially the M_n-value drastically with the set points. This strong influence of the integration limits and the baseline setpoints on the molecular weight data is one of the reasons for the limited precision and reproducibility of the molecular weight averages.

A typical value for the M_w-value of the broad polystyrene sample under the chosen conditions is:

Molecular weight	Typical GPC value	Limits (± 20%)
M_w	32000	25600 - 38400

The M_w-data should be in a range of ± 20% within the typical value. Try to come closest to the reference value. If you cannot succeed refer to Part C Appendices, chapter I. Troubleshooting.

The lab to lab reproducibility of molecular weight data depends on several hardware and software parameters as the table below shows:

Hardware Parameters	Software Parameters
• Column performance	• Accuracy of calculation procedures
• Flow precision	• Quality of baseline setting
• Temperature precision	• Quality of setting the calculation start and end marks
• Baseline stability	• Quality of calibration curve
• Injection volume precision	• Number of data points
• Quality of the standards	• Possibility to use an internal standard correction for flow rate changes

Because of the many influencing parameters lab to lab reproducibility is often poor. R. Bruessau reports [1] of an European round robin test, which resulted in differences from lab to lab for M_n of ± 16 % and for M_w of ± 9%. Similar data were reported from a Japanese round robin test[2]. In both round robbin studies only experienced GPC laboratories participated!

6. Highlight the [Mass Distribution(M)] window, then select the [Raw Data] menu and press [Print].
You will get a single page report printed consisting of the mass distribution, the main method parameters and in the bottom the molecular weight data.

7. The molar mass distribution should look like the one shown in figure 13.

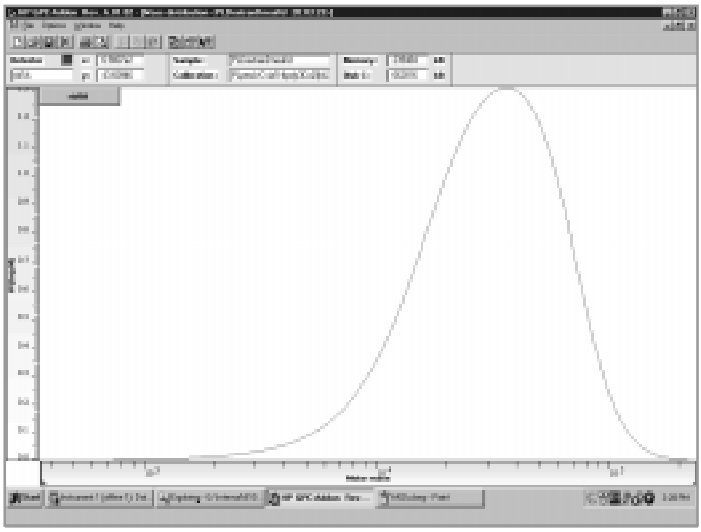


Fig. 13:
Typical molecular weight distribution of the broad dextran sample

8. Determine the molecular data in the same way for the second data file obtained with broad dextran test sample (yellow vial).

IX. Automatic calculation of molecular weight data

In this chapter is explained how to set up the system for a completely automatic analysis consisting of data acquisition, GPC data analysis and reporting based on the above developed method. An Agilent 1100 Series GPC analysis system with degasser, pump, autosampler, thermostatted column compartment and refractive index detector is required.

a) Preparation and start

1. Make sure that the system is still running using the above conditions and that method AK_SAM.M is loaded. In the [GPC Settings] window the calibration file AK_CALIB.CAL is activated.

2. Load the last data file obtained for the broad dextran test sample.

3. The integration parameters in AK_SAM.M need to be optimized for the peak shape obtained for the broad dextran with your system. The GPC data analysis part of the ChemStation calculates in a sequence automatically the molecular weight data between the start mark of the 1st integrated and the end mark of the last integrated peak. It is therefore extremely important that

   - Only the polymer peak is integrated (refer to figure 14)
   - The start and end marks for the polymer peak are placed exactly where the peak starts and ends (zoom in several times for better seeing this, refer also to chapter VIII and figure 11)
   - For optimizing the start and end marks use (refer to the ChemStation help text):

	Remarks:
• **Slope sensitivity**	*Big impact on start and end mark (use values as 0.01 or 1000 for studying th influence)*
• **Peak width**	*Big impact on start and end mark (use values as 0.01 and 10 for studying the influence)*
• **Negative peaks On**	*Negative peaks are integrated well and baseline is not drawn to minumum of negative peaks*
• **Integration On/Off**	*For suppression of peaks of no interest*
• **Baseline Now**	*Forces integrator to reset baseline*

Change the above parameters until the start and end marks are optimized. Correct drawing of the baseline itself within the standard Data Analysis is not important. The GPC data analysis itself draws the baseline arbitrarily from start to end mark.

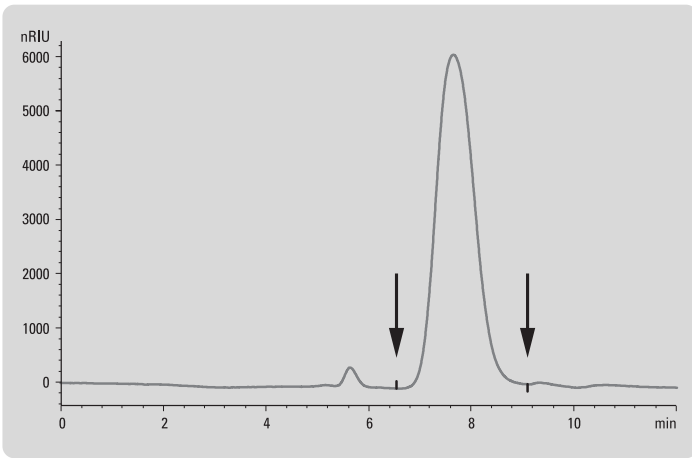


Fig. 14:
Example for broad test sample with correctly set integration marks

4. Go into the [GPC Settings…] and
   - Change the Report Settings from "Interactive Screen Review" to "Print Results"
   - Configure a report consisting of, e. g., "Graphical MWD", a "Method"- and a "Results" part
5. Save the method again and set up a sequence consisting of 6 injections of the broad dextran test sample
6. Check for baseline stability and start the sequence

b) Evaluation

1. You should have received automatically the following printouts for each run
   - A standard HPLC report
   - A single page GPC report consisting of one "Graphical MWD"-, one "Method"- and one "Results" part
2. In the ChemStation Data analyis make an overlay of the six analysis
3. The overlay should look visually like a single chromatogram, at least in the polymer region.

You may have to discard the 1st analysis to obtain repeatable chromatograms.

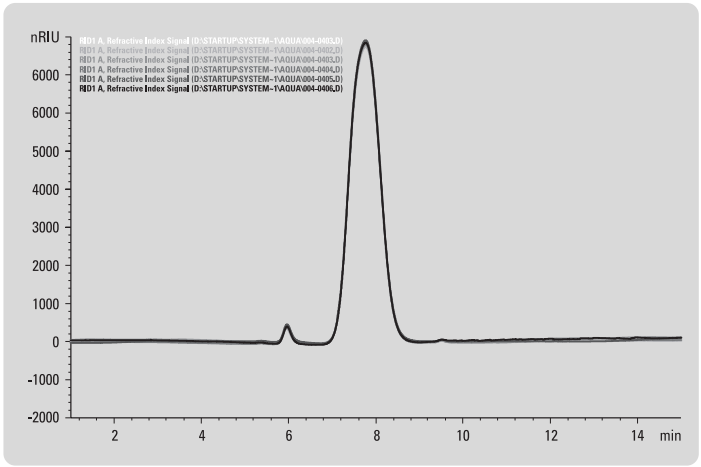


Fig. 15:
Overlay of chromatograms obtained from automatic analyses

3. Calculate the Standard Deviation (SD) and Relative Standard Deviation (%RSD) for the obtained M_n- and M_w-values using the following formulas or your pocket calculator:

$$\text{Standard Deviation} = \sqrt{\frac{n}{n-1}\sum(x-\bar{x})^2}$$

$$\%\ \text{RSD} = \frac{SD}{\bar{x}} \cdot 100$$

n = number of runs
x = actual value of M_n or M_w
$\bar{x}$ = average Value of M_n or M_w

4. The relative standard deviations should be

- For M_n < 10 %
- For M_w < 7 %

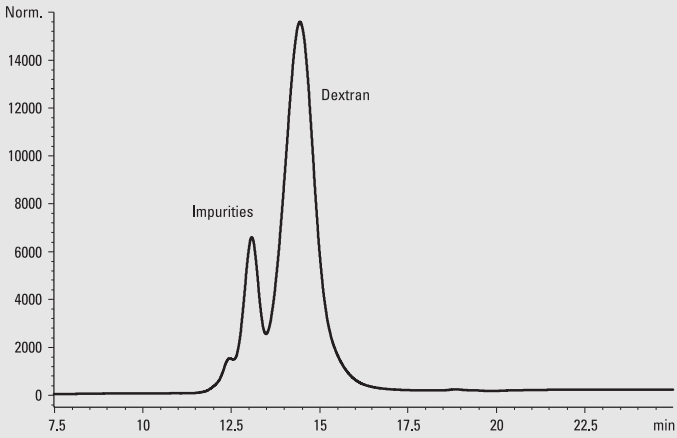
If this is not the case refer to Part C, chapter I. Troubleshooting. For more information on precision of molecular weight data achievable with optimized, state of the art equipment refer to literature 5)

X. Applications

a) Quality control of dextran

The chromatogram shows the analysis of a dextran used in cosmetic formulations.

The initial analysis with just one PL aquagel-OH Mixed column indicated already with a shoulder in the main product presence of impurities. Through the addition of one PL aquagel-OH 30 column the resolution could be further increased thus clearly proving the impurity.



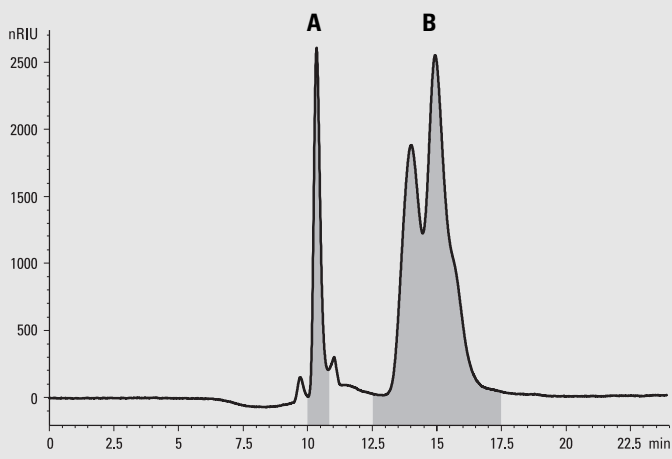
Columns:	PL aquagel-OH Mixed, 7.5 x 300 mm, 8 µm (Agilent Part No. 79911GF-MXA) in series with PL aquagel-OH 30, 7.5 x 300 mm, 8 µm (Agilent Part No. 79911GF-083)
Mobile phase:	Water
Column temp.:	25 °C
Flow rate:	1 mL/min
Detector:	Refractive Index Detector
Calibrant:	Polyethylene oxide EasyCal standards in vials for calibration

Fig. 16:
Quality control of dextran

b) Quality control of polyethoxylate

Polyethoxylates from trimethylol-propane are used in polymer industry for the production of protective coatings, resins, elastomers and as intermediates for the sythesis of other products.

The chromatogram shows the analysis of a product with a very high molecular weight part eluting between 10 and 11 minutes and a lower molecular weight part eluting between 12.5 and 17.5 minutes. Because of the bimodal molecular weight distribution the PL aquagel-OH Mixed column was used in series with one PL aquagel-OH 30 column.



Columns:	PL aquagel-OH Mixed, 7.5 x 300 mm, 8 µm (Agilent Part No. 79911GF-MXA) in series with PL aquagel-OH 30, 7.5 x 300 mm, 8 µm (Agilent Part No. 79911GF-083)
Mobile phase:	Water
Column temp.:	25 °C
Flow rate:	1 mL/min
Detector:	Refractive Index Detector
Calibrant:	Polyethylene oxide EasyCal standards in vials for calibration

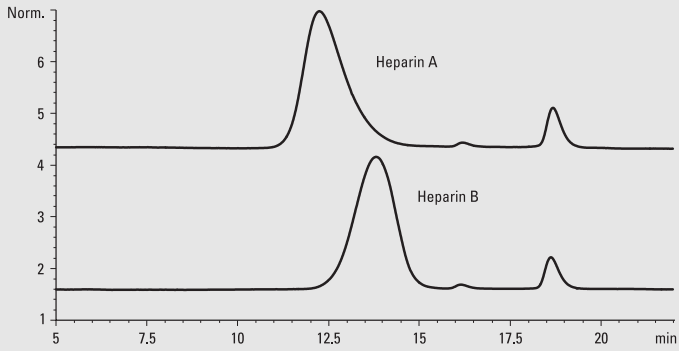
Fig. 17:
Quality control of polyethoxylate

Summary of results:

Part	M_n	M_w
A	1920000	2002000
B	4000	6000

c) Analysis of heparins

Heparins are mucopolysaccharides formed by the reaction of D-glucoseamines and D-glucuronicacid. They are widely used as anticoagulants to prevent thrombolysis in case of hyperlipidemia, arteriosclerosis, blood transfusions and operations. Their properties depend on the molecular weight and the molecular weight distribution which can be fast and precisely controlled by aqueous GPC-SEC.



Column:	2 x PL aquagel-OH 30 in series, 7.5 x 300 mm, 8 µm (Agilent Part No. 79911GF-083)
Mobile phase:	0.2 M $NaNO_3$, NaH_2PO_4, pH 7
Column temp.:	25 °C
Flow rate:	1 mL/min
Detector:	Refractive Index Detector
Calibrants.	Polysaccharides (Part No. 1535-4546) (10 x 0.2 g)

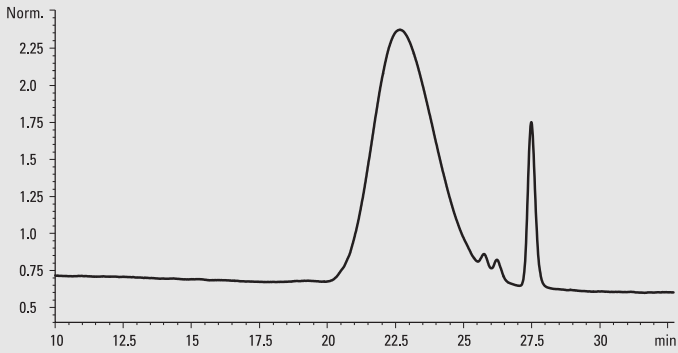
Fig. 17:
Analysis of heparins

Summary of results:

Part	M_n	M_w
A	22500	28200
B	9350	10770

d) Analysis of polyvinyl alcohol

Polyvinyl alcohols are industrially synthesized by the catalytic reaction of polyvinyl acetates with alcohols, typically methanol. Due to properties as excellent biological degradeability, water solubility , toxilogical harmlessness they are widely used as emulgators, binding agents, in adhesives, salves, haircream. The properties can be varied with the molecular weight distribution and the molecular weight which ranges from 20000 to 100000 g/mol.



Columns:	3 x PL aquagel-OH 30 in series, 7.5 x 300 mm, 8 µm (Agilent Part No. 79911GF-083)
Mobile phase:	0.2 M $NaNO_3$, NaH_2PO_4, pH 7
Column temp.:	25 °C
Flow rate:	1 mL/min
Detector:	Refractive Index Detector

Fig. 18:
Analysis of polyvinyl alcohol

For more application examples refer to "Polymer and Hydrocarbon Processing Solutions Guide", Agilent publication number 12-5968-7020E.

C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5
C_6H_5

Part C

Getting Ready for GPC-SEC Analysis

Appendices

I. Troubleshooting

Problem:	Possible Causes:	Solution:
1. Unstable retention times	Column not well conditioned	Flush with eluent
	Leak	Search for leak and remove it
	Air bubbles in eluent	Defective degasser pump as indicated by red error lamp
	Defective pump seals	Replace seals
	Column temperature not stable	Check column thermostat
2. Consecutive injections from the same standards and/or the broad test vial do not overlay well	Column adsorbs polymer, column not well conditioned	Flush with eluent and inject again until o.k., GPC-SEC columns often adsorb polymers until an equilibrium is achieved
	Pump flow not stable	Refer to 1.)
	Column temperature not stable	Refer to 1.)
	Injection volume not precise	Rotor seal defective Metering seal
	Standards/samples not completely dissolved	Refer to chapter IV. Preparation
3. Molecular weight data M_w not within ± 20% of typical value	Flow precision not good: the system must easily perform the factory specification < 0.3%	Leak (refer to 1.) Air bubbles in eluent (refer to 1.) Defective pump seals
	Column temperature precision not good: thermostat should perform the specification: ± 0.15 °C	Switch thermostat off and on Perform thermostat test from Diagnosis part of ChemStation
	Detector baseline: drift and noise too high, exact setting of baseline and calculation start/end marks not possible	Flush sample and reference cell for 10 minutes Activate "Recycle" from the RI control menue for several minutes Column not well conditioned Optical unit temperature not stable
	Quality of standards	Prepare standards newly for each calibration
	Quality of broad sample	Prepare samples newly for measurement
	Defective column	Handle column as described in the PLgel column user's guide Determine plate number and symmetry according to test chromatogram supplied with the column

Problem:	**Possible Causes:**	**Solution:**
3. Molecular weight data M_w not within ± 20% of typical value	Baseline not set correctly Calculation start and end marks not set correctly	Repeat calculation as explained in chapter VII
	Calibration curve fit not optimized	Optimize curve fit as explained in chapter VI
	Wrong calibration curve in use	Select correct calibration curve
4. Relative standard deviation for M_n not < 10%, for M_w not < 7%	Flow precision not good: the system must easily perform the specification < 0.3%. A typical, well maintained GPC system has a retention time precision < 0.1%	Leak (refer to 1.) Air bubbles in eluent (refer to 1.) Defective pump seals
	Column temperature precision not good: thermostate should perform the specification: ± 0.15 °C	Switch thermostat off and on Perform thermostate test in diagnosis and OQ/PV parts of ChemStation
	Detector baseline: drift and wander too high, repeatable setting of baseline start/end marks not possible	Flush sample and reference cell for 10 minutes to get air bubbles out, use recycle mode for several minutes for better flushing Column not well conditioned Optical unit temperature not stable
	Baseline start and end marks not set repeatable	Optimize integration parameters as outlined in chapter IX. Automatic Calculation of Molecular Weight Data.
5. Unknown peaks elute before the volume of total exclusion and after the volume of total penetration	Column impurities from previous application elute	**Columns for GPC-SEC with organic eluents:** clean column by flushing thoroughly with THF **Columns for SEC with aqueous eluents:** clean column by flushing thoroughly with water. If this does not help, add up to 50% methanol
	Degradation of standard/ test sample	Prepare standards and test sample always newly
	Baseline wander of refractive index detector too high	Purge reference cell Activate recycle mode for better flushing

II. Literature

(1)
R. J. Bruessau, "Experiences with Interlaboratory GPC Experiments", Maromol. Symp. 110, 15-32 (1996)

(2)
The Japan Society for Analytical Chemistry, Bunseki Kagaku 44(6), 497-504 (1995)

(3)
DIN 55672-1 Gelpermeationchromatography (GPC), Teil 1: Tetrahydrofuran (THF) als Elutionsmittel, Berlin 1994

(4)
ISO/TC35/SC N 950 = ISO CD 13885 1994-05-8 Gel permeation chromatography

(5)
H. Goetz, H. Schulenberg-Schell, "Improved Precision of Molecular Weight Data using advanced GPC procedures", submitted for publication
"Int. Journal of Polymer Analysis and Characterization".

Recommended books

(1)
"Size Exclusion Chromatography", edited by B. J. Hunt and S. R. Holding, Blackie and Son Ltd.

(2)
"Handbook of Size Exclusion Chromatography" edited by Chi-san Wu, Marcel Dekker inc.

III. Acknowledgement

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