Agilent GPC Data Analysis Software for Agilent ChemStation

Installing and Understanding your GPC Data Analysis Software

Agilent Technologies

Notices

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This guide is valid for B.01.01 revisions of the Agilent GPC Data Analysis Software for Agilent ChemStation software,

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In This Guide...

This manual will help you to familiarize yourself with the Agilent Technologies GPC data analysis software. Further, the manual describes in detail all the features and evaluation parameters of the software. The index at the end of the manual allows to search easily for certain keywords and gives quick access to necessary information. There is also extensive online help available within Agilent ChemStation and the GPC data analysis software. The manual contains the following information:

1 Installing the GPC Data Analysis Software

Requirements and procedures to install or uninstall the Agilent GPC data analysis software

2 Basic Theory of GPC

The importance of gel permeation chromatography (GPC) in polymer characterization has strongly increased since its introduction by Moore[1] and others. Quality assurance and control—even during production and processing stages—have increased in significance to retain international competitive ability. Also, exact knowledge of molecular weights and their distribution is important in polymer research in academia and industry.

However, support for users in evaluation of GPC data has not found the necessary interest in the past. The improvement of GPC software has not kept pace with improvements and redevelopments of GPC and liquid chromatography (LC) components. As a result even to today GPC data often have to be evaluated manually.

Through the increasing automation of polymer analysis, GPC software today should be both flexible and powerful and simplify the workload of the user, making effective use of powerful and expensive hardware.

GPC is characterized by a series of specific marginal conditions, some of which differ considerably from the conditions of LC:

- completely different calibration procedure for transformation of elution volume in molar masses
- completely different separation mechanism—diffusion controlled exclusion instead of adsorption equilibration
- basically different requirements to the system parameters which determine the reproducibility, measurement and evaluation accuracy

- variety of time bases—measurement time, peak width, and so on
- variety of analysis goals—molecular mass specification instead of qualitative or quantitative analysis
- multidetection in GPC suggests parallel evaluation of all used detectors
- evaluation procedure should automatically process volume correction between the different detectors, so that only one calibration relation will be needed for all connected detectors

3 Introduction to the GPC Data Analysis Software

The Agilent GPC data analysis software integrates seamlessly into your existing Agilent ChemStation software and adds GPC data analysis features to it. The product directly uses the Agilent ChemStation support for Agilent instrumentation and fully utilizes its data acquisition and sample handling features. The Agilent GPC data analysis software supports conventional GPC analyses with multiple calibration methodologies:

- narrow standard calibration
- universal calibration
- broad standard calibration
- integral calibration
- automated recalibration

Data processing is limited to concentration detectors (like UV, DAD, RID or any signal detector connected via Agilent 35900E A/D converter); it does not handle light-scattering or viscometric detection, nor does it support special GPC evaluation strategies like copolymer analysis and chemical heterogeneity studies. Users who need that functionality should look for specialized GPC data systems. Agilent GPC data analysis software offers 3 different modes of GPC data processing:

- automatic GPC data processing for single runs and sequences (on-line and off-line)
- manual reprocessing of data files from the standard Agilent ChemStation data analysis
- interactive reprocessing of GPC runs for fine-tuning demanding samples in the Agilent GPC data analysis software window.

The Agilent GPC data analysis software is capable of multitasking and can run these modes in parallel. While one sequence of samples is analyzed and new data are acquired and reported, stored data files can be loaded and

reprocessed starting from the standard Agilent ChemStation data analysis or from the interactive Agilent GPC data analysis software screen using different calibration files.

This tutorial describes briefly the tasks to set up a narrow standard calibration and run samples and recalibration standards using a single detector configuration. For further reference go and find details in Chapter 4, “Interactions Between Agilent ChemStation and the Agilent GPC Data Analysis Software and Chapter 5, “Software Windows and Menus. This section requires that you are familiar with operating the standard Agilent ChemStation.

4 Interactions Between Agilent ChemStation and the Agilent GPC Data Analysis Software

This chapter describes the how to acquire and process data files starting from the Agilent ChemStation Data Analysis view. The individual tasks and menu items are described in detail.

5 Software Windows and Menus

This chapter describes all windows and menu items of the Agilent GPC data analysis software window.

The menu bar appears on the upper edge of the display regardless of which window is selected. The possible menu options are context specific that means that each active window has its own menu entries. Only the menu item window does not change irrespective of the currently active window.

6 Appendix

This chapter provides supplementary information about the software such as system verification.

7 References

This chapter gives the literature references quoted in the manual.

Contents

1 Installing the GPC Data Analysis Software

Minimum Requirements for the Agilent GPC Data Analysis Software (32bit version)

This section specifies the PC hardware, operating system and Agilent ChemStation software requirements that must be met for successful installation and operation of the Agilent GPC data analysis software, revision B.01.01.

Operating System

The Agilent GPC data analysis software is supported on Window XP.The Agilent GPC data analysis software is not supported on Windows 95, Windows 98, Windows 2000 and Windows ME.

Minimum PC Configuration

Agilent recommends that you order the PC on which you run the Agilent ChemStation software as a bundled system. Agilent carefully selects PC's that can be used reliably in a typical laboratory operation mode.

The minimum PC configuration depends on the revision of the Agilent ChemStation software and the Windows operating system. The following requirements are valid for Agilent ChemStation revision B.02.01. For further information refer to the Readme-text on your Agilent ChemStation Software CD.

Requirements Windows XP based systems:

- Hewlett-Packard / Compaq PC with Pentium IV*, 1.5 GHz
- XGA display (1080x1024 resolution)
- 40 GB hard disk
- MS Windows compatible pointing device
- ATAPI CD, CD-RW or DVD drive
- 10/100 baseT LAN interface card.

Minimum memory specifications:

- 512 MB RAM

When using the Agilent ChemStation GPC data analysis software a maximum of two HPLC instruments operated from one PC can be used. The number of HPLC instruments is limited to one when an LC/MSD ChemStation is used.

For further details of PC hardware, see the *Installing Your Agilent ChemStation* manual.

Printers for the Agilent GPC Data Analysis Software

For routine operation we recommend to use the printer and printer drivers delivered with the Agilent ChemStation, as they have been tested to work reliably with the Agilent ChemStation. You can also find a list of printers in the *Installing Your Agilent ChemStation* manual.

Installing the Agilent GPC Data Analysis Software

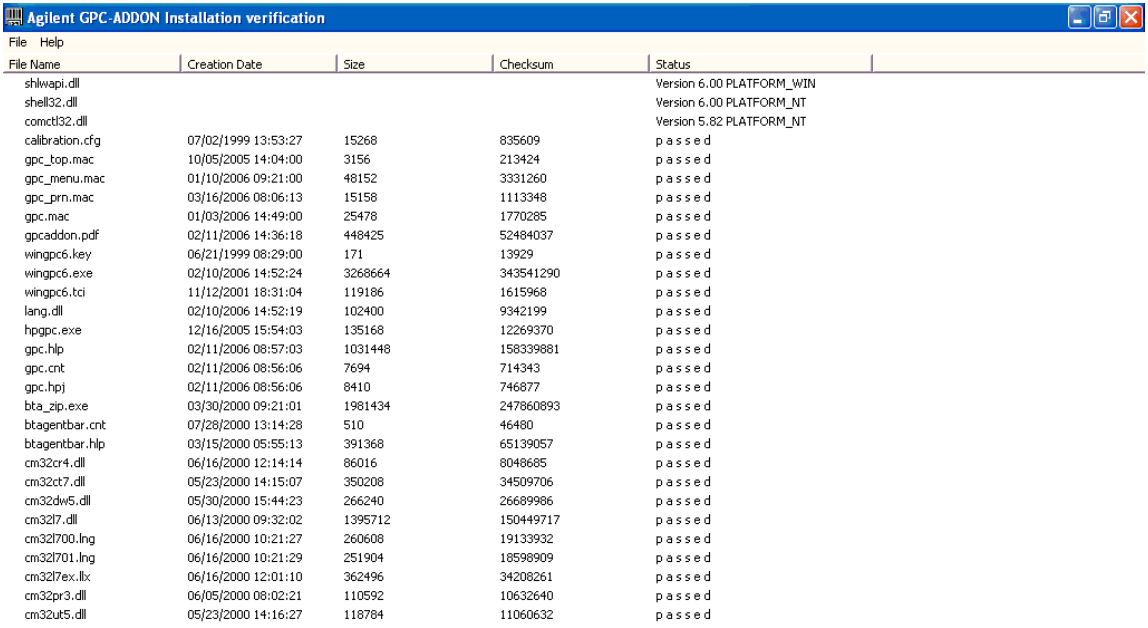
This section gives a step-by-step description of how to install the Agilent GPC data analysis software.

1. Install the Agilent ChemStation for 2D LC, 3D LC, or LC/MSD systems as described in the *Installing Your Agilent ChemStation* manual, if not already done.
2. Start the Agilent ChemStation to check for proper operation, then close it.
3. Insert the Agilent GPC data analysis software CD-ROM into the CD-ROM drive.
4. Select Run from the Start menu and type `E:\setup.exe` where `E` is the CD-ROM drive.
5. Read and follow the instructions on the screen.
   An important notice points out that a maximum of 2 instruments controlled by one PC are supported when using the Agilent GPC data analysis software (with LC/MSD ChemStation only 1 HPLC instrument is supported) .
6. Select the instrument you want the Agilent GPC data analysis software installed for. If you have 2 instruments installed, you need to repeat the installation process for the second instrument.

NOTE With the LC/MSD ChemStation only GPCaddon installation is supported.

Installation Verification

To verify correct installation of the core components of the GPC data analysis software, you can run the utility Instveri.exe located in the CHEM32\GPC directory. All files should be listed as passed.



File Name	Creation Date	Size	Checksum	Status
shlwapi.dll				Version 6.00 PLATFORM_WIN
shell32.dll				Version 6.00 PLATFORM_NT
comctl32.dll				Version 5.82 PLATFORM_NT
calibration.cfg	07/02/1999 13:53:27	15268	835609	passed
gpc_top.mac	10/05/2005 14:04:00	3156	213424	passed
gpc_menu.mac	01/10/2006 09:21:00	48152	3331260	passed
gpc_prn.mac	03/16/2006 08:06:13	15158	1113348	passed
gpc.mac	01/03/2006 14:49:00	25478	1770285	passed
gpcaddon.pdf	02/11/2006 14:36:18	448425	52484037	passed
wingpc6.key	06/21/1999 08:29:00	171	13929	passed
wingpc6.exe	02/10/2006 14:52:24	3268664	343541290	passed
wingpc6.tci	11/12/2001 18:31:04	119186	1615968	passed
lang.dll	02/10/2006 14:52:19	102400	9342199	passed
hpgpc.exe	12/16/2005 15:54:03	135168	12269370	passed
gpc.hlp	02/11/2006 08:57:03	1031448	158339881	passed
gpc.cnt	02/11/2006 08:56:06	7694	714343	passed
gpc.hpj	02/11/2006 08:56:06	8410	746877	passed
bta_zip.exe	03/30/2000 09:21:01	1981434	247860893	passed
btagentbar.cnt	07/28/2000 13:14:28	510	46480	passed
btagentbar.hlp	03/15/2000 05:55:13	391368	65139057	passed
cm32cr4.dll	06/16/2000 12:14:14	86016	8048685	passed
cm32ct7.dll	05/23/2000 14:15:07	350208	34509706	passed
cm32dw5.dll	05/30/2000 15:44:23	266240	26689986	passed
cm32l7.dll	06/13/2000 09:32:02	1395712	150449717	passed
cm32l700.lng	06/16/2000 10:21:27	260608	19133932	passed
cm32l701.lng	06/16/2000 10:21:29	251904	18598909	passed
cm32l7ex.llx	06/16/2000 12:01:10	362496	34208261	passed
cm32pr3.dll	06/05/2000 08:02:21	110592	10632640	passed
cm32ut5.dll	05/23/2000 14:16:27	118784	11060632	passed

In addition to the Agilent GPC data analysis software, the files and methods listed in Table 1 were copied to your system.

Table 1 Additional Data Files

Name	Location	Description
GPC_DEMO	CHEM32\1\DATA	Demo Agilent ChemStation data files
Gpc_sam.m	CHEM32\1\METHODS	Demo Agilent ChemStation method file for samples
Gpc_stan.m	CHEM32\1\METHODS	Demo Agilent ChemStation method file for standards
Gpc_demo.s	CHEM32\1\SEQUENCE	Demo Agilent ChemStation sequence
default.cal demo_gpc.cal	CHEM32\GPC\CALIB	Demo Agilent GPC data analysis software calibration files
AK_STAN.M AK_SAM.M OK_STAN.M OK_SAM.M	CHEM32\1\METHODS	Methods files for GPC-SEC start-up kits "Getting ready for GPC-SEC Analysis", PN: 5064-8251 (organic kit) and 5064-8252 (aqueous kit)

Uninstalling the Agilent GPC Data Analysis Software

The GPC Data Analysis Software can be uninstalled from the "Add Remove Programs"utility which is accessible in the Windows "Control Panel". Please note that the uninstall procedure does NOT remove the installed demo files. The demo calibration files are stored in <ChemStation directory>\GPC\calib. To fully remove the GPC Data Analysis Software GPC-Addon you have to manually remove the GPC directory after you have saved your calibration files at a different location.

If you have two instruments in your GPC system you must uninstall them in the reverse order of their installation. Make sure that you first uninstall the second installation! The order of installation can be determined from the Add/Remove Programs screen. The list is sorted according to the order of installations. Perform the un-installation from the bottom to the top.

Maintaining the Agilent GPC Data Analysis Software

On every computer system, regular maintenance is mandatory to ensure correct operation. Temporary files and configuration settings of the Agilent GPC data analysis software are stored in the directory \HPWINGPC. Usually the files in this directory do not need to be changed. If there are problems during startup of the Agilent GPC data analysis software, this directory can be deleted to reset the Agilent GPC data analysis software.

No further maintenance steps are required for the Agilent GPC data analysis software, except for the general steps described in the manual *Configure and Maintain Your Agilent ChemStation Computer*. These steps are:

- Scanning for viruses.
- Performing backups
- Cleaning up left-over temporary files

We strongly recommend that you adhere to the maintenance tasks described in the manual *Configure and Maintain Your Agilent ChemStation Computer* manual to ensure proper operation of the Agilent GPC data analysis software.

2
Basic Theory of GPC

Basics of Gel Permeation Chromatography

In contrast to gas chromatography (GC) and LC the separation mechanism in GPC is not based on a distribution equilibration—it is based on volume exclusion.[2] The separation results in GPC are achieved due to the hydrodynamic volume, V_h, of the sample molecule.[3] This can be determined from pseudo-elastic, light-scattering experiments measuring the diffusion coefficients. The separation in GPC is also based on the molecular size and not on the molecular mass of the sample molecule.

Macro-porous polymer gels are generally used as column material in GPC. The diffusion of molecules between mobile phase and pore is the basis for separation mechanism and performance. Since for smaller molecules more pores are accessible, these molecules are more strongly retarded and consequently elute later than the higher molecular fractions.

Unfortunately GPC does not present an absolute method, that is, the retention times (called elution volume, V_e, in GPC terminology) have no direct relation to molecular mass of the examined substance and depend on the measurement conditions (type of polymer, columns, solutions, and so on.)

Therefore an accurate calibration is necessary within the used analytical conditions. Under optimum conditions it is possible to determine molecular weights quickly, economically and reliable, with an accuracy that does not differ from other absolute methods (for example, osmosis or light scattering.) Differing from these techniques GPC does not have high demands for sample preparation. Additionally to the molecular mass GPC also yields the molecular weight distribution, which influences many physical and physico-chemical properties and therefore is of great interest.

Molecular Weight Averages and Mass distributions

The calculation of the molecular weight averages nowadays uses the slice method. Hereby the eluted peak is separated into several equidistant volume slices. Through calibration the elution volume is then transformed into the molecular mass.

In calculating the molecular averages the slice concentrations, c_i, must be corrected with the slope of the calibration curve. This is necessary because data recording is done linearly but molecular mass however does not increase in a linear fashion. Objectively this means, that with the same concentration the number of polymer chains with a defined molecular weight (M) on the high-molecular part of the elugram is much smaller than on the low-molecular part.

The errors caused through this will increase, the broader the sample is distributed, and the smaller the data recording frequency. Only with strictly linear calibration curves the correction is not needed.

The Mass distribution w(M) can be calculated from the detector signal, $S(V_e)$ and is most important to characterize polymers. In contrast the molecular weight averages describe only average properties of the sample. For example, the molecular weight average of two samples can be identical, although the molar mass distribution is different.

The differential distribution, w(M), of the molar mass M is defined as:

$$w(M) = \frac{dm}{dM}$$

where m is the total mass.

By transformation w(M) can be expressed by measured quantities:

$$w(M) \propto \frac{S(V_e)}{M(V_e) \cdot \sigma(V_e)};$$

$S(V_e)$ detector signal

$\sigma(V_e)$ slope of calibration curve

The above introduced qualitative correction by the gradient of the calibration curve can now be allocated by the mathematical derivative of the calibration curve.

The integral distribution I(M) will be used as normalization condition resulting from:

$$I(M) = \int_0^M w(M')\mathrm{d}M'$$

The molecular weight averages can be calculated from the moments, μ_i, of the molar mass distribution:

$$\mu_i = \int_0^\infty M^i \cdot w(M)\mathrm{dM}$$

μ_i i-th moment of mass distribution

which validate the following definitions:

Number average molecular weight:

$$M_n = \frac{\sum h(M) \cdot M}{\sum h(M)} = \frac{\sum w(M)}{\sum w(M)/M} = \frac{\mu_0}{\mu_{-1}}$$

Weight average molecular weight:

$$M_w = \frac{\sum h(M) \cdot M^2}{\sum h(M) \cdot M} = \frac{\sum w(M) \cdot M}{\sum w(M)} = \frac{\mu_1}{\mu_0}$$

z- average molecular weight:

$$M_z = \frac{\sum h(M) \cdot M^3}{\sum h(M) \cdot M^2} = \frac{\sum w(M) \cdot M^2}{\sum w(M) \cdot M} = \frac{\mu_2}{\mu_1}$$

Viscosity average molecular weight:

$$M_v = \left(\frac{\sum w(M) \cdot M^a}{\sum w(M)}\right)^{1/a} = \left(\frac{\mu_v}{\mu_1}\right)^{1/a}$$

The width of the Mass distribution is described by the polydispersity index D or the non-uniformity U:

$$D = \frac{M_w}{M_n}; \text{ or } U = D - 1$$

Calibration

Only by proper calibration the elution volume can be transformed into molar masses. The calibration can be carried out in various ways:

- narrow standard calibration—simple and most frequently used
- universal calibration—if no standards are available for this polymer type
- broad standard calibration—if broad standards are available for this polymer type
- integral calibration—requires only one broad standard

Calibration with Polymer Standards of Narrow Mass distribution

The use of polymer standards with narrow mass distribution is the simplest way to assign molecular weight to elution volume. For this it is best to assign M_p-values (molar mass on the peak maximum), as this value is the only molecular weight that can clearly be identified in the elugram. If weight average molecular weights, M_w, are used, then the calibration function should be iterated until the used M_w-values will be received again by recalculation.

Calibration using Universal Calibration

Since for some polymers no molecular weight standards are available, very early possibilities were studied to convert existing calibrations for use with other types of polymers. This method developed by Benoit[4] is based on the assumption, that the property that determines the elution behavior in GPC is

the hydrodynamic volume, V_h, of the polymer under investigation. Since V_h should be proportional to the product of intrinsic viscosity, [η], and molecular weight, M, it follows for two polymers eluting at the same elution volume:

$$[\eta_1] \cdot M_1 = [\eta_2] \cdot M_2 \text{ at same elution volume}$$

Using the Mark-Houwink relation

$$[\eta] = K \cdot M^a \text{ where } a \text{ and } K \text{ are Mark-Houwink constants.}$$

The molecular weight of the polymer type 1 can be converted into the molecular weight of polymer type 2.

$$\lg M_2 = \frac{1}{1 + a_2} \lg \frac{K_1}{K_2} + \frac{1 + a_1}{1 + a_2} \lg M_1$$

It is apparent, that the Mark-Houwink constants must be known for both polymers in the respective eluent under separation conditions.

Unfortunately this is not the case for frequently used GPC eluents (for example, THF, DMF). Viscosity measurements are then necessary to obtain the Staudinger Indices. However, these determinations of intrinsic viscosities for some polymers are difficult in some solvents (for example, PMMA in THF).

In such cases a workaround may help. Instead of viscosity measurements the polymer standards with known weight average molecular weight (M_w) are characterized by GPC. V_p is determined and [η] M(V_p) is calculated from the calibration curve of a polymer, whose Mark-Houwink constants are known. Thus, it is possible to determine intrinsic viscosities of polymers by GPC.

By plotting of log [η] against log M of the polymers investigated by GPC the Mark-Houwink coefficients are calculated from slope and intercept.

If there are no standards for your polymer type, the samples have to be fractionated, if you want to use this calibration method. In addition the weight average molecular weight (M_w) from light scattering measurement must also

be determined. The viscosity measurement for the determination of the Staudinger-Index can be renounced, if this method for calculation of the intrinsic viscosity is used.

NOTE

The Mark-Houwink relation is valid only above a certain molecular weight, which depends on the polymer type (about 10000 to 20000 D). Below this limit the Mark-Houwink coefficients are dependent on the degree of polymerization. In this case the plot of log [η] against log M deviates from the straight line to higher viscosities. The reason for this non-linearity is based on a change of the structure. It usually changes from a worm-like type to a Gaussian coil structure.

The universal calibration presents a very useful calibration method; its validity should be checked for the used polymer type (for example, literature, combination of direct calibration with universal calibrated samples). Special attention should also be paid working in the molar mass section below about 20000 D.

Calibration with Broad Standards

The calibration procedure used by the Agilent GPC data analysis software is based on papers of Mahabadi[5], Weiss[6] and Mori[7], which use the dependence of the GPC-separation on hydrodynamic volume, to calibrate reliable and flexible with broad standards. The procedure described here has no limitations (for example, only linear calibration function or only calibrations with inaccurate M_n and M_w) and even permits deduction of the Mark-Houwink coefficients of the polymers under investigation. Because this calibration method is based on the universal calibration, their requirements have to be considered as well.

These are the requirements for establishing such a calibration:

1. Existence of a base calibration curve (Index: 1), which will be used for the characterization of the pore size distribution of the column set used.
2. One or more broad polymer samples (Index: 2) with known average molecular weight values (M_n and/or M_w and/or [η]), which must be clearly known (valid for all broad calibration procedures).

According to the theory of universal calibration for each elution volume and independent of the type of polymer it is necessary, that all samples have the same hydrodynamic volume. The molecular weight on the other hand may be

different, however a transformation can be carried out (see universal calibration), which depends on the stiffness of the main chains of the considered polymer.

For each elution volume the following equation holds true:

$$M_2 = A \cdot M_1^B$$

whereas A and B are constants, which must be optimized through the mean values of the wide samples.

In order to do so, A and B are varied and the molecular weight averages are calculated from the elugrams and the calibration curve. These molecular weight averages are compared with the reference values. This process will be optimized with a Simplex-algorithm until the calculated and reference molecular weights agree sufficiently exact.

The calculated A- and B-Values correspond to the hydrodynamic parameters as follows:

$$A = \left(\frac{K_1}{K_2}\right)^{\frac{1}{1+a_2}} \text{ and } B = \frac{1+a_1}{1+a_2}$$

K, a: Mark-Houwink constants for basic polymer 1 and wide sample 2

If Mark-Houwink constants of polymer 1 are known under the conditions used, the Mark-Houwink constants for the unknown sample can be calculated.

Independent of the procedure the user always has the problem, to optimally adapt the measured or calculated calibration points with a function. Since the calibration function by GPC is not linear or only slightly curved as usual for GC or LC, but shows a definite S-shaped curvature, it is difficult to describe this dependence by a simple function. Only dedicated GPC software offers a suitable solution.

Flow Correction and Internal Standard

Besides choosing the appropriate calibration procedure the reproducibility of the analysis conditions plays an important role. Although GPC instruments can achieve very good reproducibility, GPC requires special data recording and processing. The accuracy of the elution volume is determined through the quality of the constant flow pump. Here GPC again shows deviations from well-known LC conditions. Because the separation of GPC occurs through a diffusion-controlled process in porous polymer gels, the thermodynamic condition of the gel in the column also highly influences the reproducibility of the separation.

Users have already observed this effect. The equilibration of GPC columns takes much longer than the time needed by the pump to produce a constant flow and analysis in this phase clearly yields different results than the analysis after complete equilibration of GPC columns.

Both factors can be easily registered by use of an internal standard, which should be added to the sample solvents. Often lower molecular substances are used as internal standard, which are specifically distinguished through their characteristics (absorption, refraction, and so on). Use the same internal standard in calibration and sample run. The retention times/elution volumes are then corrected for deviations.

A modification of the gel condition and the flow is reflected in the displacement of the internal standard. Upon correcting the experimental elution volume by aid of the volume of the internal standard for analysis and calibration, such effects can be balanced. Using this procedure it is easier to use calibration libraries and save the time consuming calibrations prior to each sample series. The concept of using internal standards requires a continuous change of the gel condition and/or of the flow—erratic changes can not be corrected.

The corrected elution volume is calculated by:

$$V_e^{adj.} = V_e^{analysis} \cdot \frac{V_{\text{int. Std.}}^{calibration}}{V_{\text{int. Std.}}^{analysis}}$$

The use of this correction also permits to determine GPC molecular weights exactly and reproducible. Considering the requirements of light scattering measurements or other absolute methods like membrane osmosis or ultra centrifugation, GPC is very qualified for routine type characterization of polymers.

In order to demonstrate the influence of the correction with the internal standard, the mass distribution of a Poly(o-Chlorostyrene) is shown in Figure 1 on page 28, which has been evaluated with (solid curve) and without correction (dotted curve) for the internal standard (BHT; measurement in THF by room temperature with 1.0 ml/min.). The change of the weight average, M_w, is more than 10%, however the difference in elution volume between reference and experimental elution volume is less than 1% (20.37 against 20.20 ml). These deviations become even more pronounced, if the slope of the calibration curve becomes steeper.

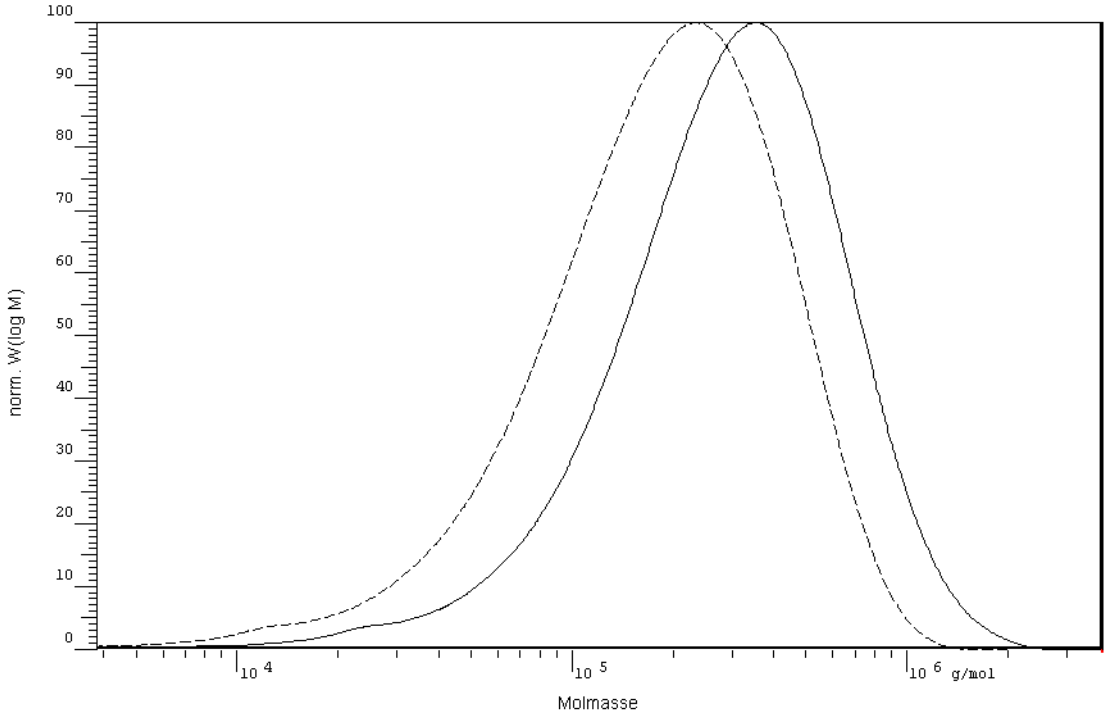


Figure 1 Influence of Volume Correction of the Internal Standard on the Mass distribution

How much this fluctuation of the flow and/or modifications of the gel condition influence the molecular weights is also shown in Table 2 on page 29.

Table 2 Influence of the Internal Standard Position on the Molecular Weights, for example, on Weight Average Molecular Weight M_w

Internal Standard El. Volume [ml]	Difference in%	M_w in D	Difference in%
21.80	+ 2.01	46.500	+ 36.8
21.65	+ 1.31	41.900	+ 23.2
21.50	+ 0.61	37.500	+ 10.3
21.	-	34.000	-
21.20	- 0.80	29.800	- 12.4
21.05	- 1.50	26.400	- 22.4
20.90	- 2.20	23.300	- 31.5

These deviations not only show up in the Mass distribution, but also in the elution curves, however not so clear. The values mentioned in the table generally depend on the used column combination and the position of the peaks in the calibration curve. For linear (mixed bed) columns or columns with higher particle size (>10 µm) but same column length a higher deviation must be taken into consideration. Also the preferred overlay of elugrams and Mass distributions will be more evident by use of an internal standard.

Determination of Detector Delay

Agilent GPC data analysis software enables the simultaneous recording and evaluation of several detectors, for example, UV and RI detector. In this case it is not necessary to create an own calibration curve for each detector, since the chromatographic delay between the detectors is corrected on-line. To define the delay between the detectors you start with a delay of 0 ml in the Agilent GPC data analysis software method for all detectors. You inject a monodisperse substance, which yields sufficient signal intensities in the various detectors.

When you evaluate the sample in all channels you can read in the mass distribution window the V_P-values for the elution volumes at peak maximum for the different detectors. The difference of

$$\Delta V = V_P^{Detector_i} - V_P^{Detector_1}$$

is the required delay for the i-th detector.

When ΔV is determined the time difference Δt can be calculated with

Δt = ΔV/ flow rate

According to this definition the first detector has a delay of 0 min.

Separation Efficiency, Resolution and Plate Count

A simple method for controlling the performance of GPC setup is the review of the plate count. Therefore a lower molecular substance (acetone, BHT, and so on) will be injected. The details stated in the Agilent GPC data analysis software comply with ISO 13885 and DIN 55672.

The specification of the theoretical plate count per meter, N_{th}, uses the Peak position and the peak width at half the peak height according to:

$$N_{th} = \left(\frac{V_P}{\sigma}\right)^2 = \frac{554}{L[cm]}\left(\frac{V_P}{W_{1/2}}\right)^2 \qquad [m^{-1}]$$

where is σ the variance, which can be estimated of the halve width, $w_{1/2}$. L is the column length in cm.

Experimental conditions required: injection volume ≤ 20 µl, flow 1 ml/min., concentration about 50 ppm.

The peak asymmetry is defined as:

$$A = w_l / w_r$$

whereas w_l and w_r are the peak widths on the left and right side of the peaks (measured in 10% of the peak height). The definition used corresponds to DIN 55672 and ISO 13885. In LC asymmetry is defined as A'= w_r/w_l thus, A'=1/A.

The specification of the resolution has a higher importance, since it yields information about the usefulness of the column resolution. Therefore a mixture of polymer standards will be injected. The resolution R_s is calculated according to:

$$R_s = \frac{V_2 - V_1}{2 \cdot (\sigma_1 + \sigma_2)} = \frac{\lg(M_1/M_2)}{2 \cdot D \cdot (\sigma_1 + \sigma_2)}$$

with D of slope of the calibration curve.

The resolution thus defined depends apparently on the selection of used molecular weights. The specific solution R_{sp} can be defined as:

$$R_{sp} = \frac{R_s}{\lg(M_1/M_2)} = \frac{0,579}{\sigma \cdot D}$$

It specifies the quality of resolution of two peaks, whose molecular weight differs by one order in magnitude.

Within ISO 13885 and DIN 55672 it is required, that within the section of peak maximum of the sample the following conditions for the separation efficiency apply:

$$\frac{V_e(M) - V_e(10 \cdot M)}{\text{area of column cross section}} > 6[cm]$$

i.e. the separation distance between both peaks must be a minimum of 6 cm.

A value of 1.7 shows that a baseline separation of two polymers with a molar mass difference of 10 is obtained.

3
Introduction to the GPC Data Analysis Software

Overview

The Agilent GPC data analysis software is initiated from the Agilent ChemStation Data Analysis view. Locate the GPC menu and use the Switch To GPC command to switch to the Agilent GPC data analysis software. The Agilent GPC data analysis software will coexecute with the Agilent ChemStation data analysis and four windows open:

- Raw data window—showing raw data loaded from the file system
- Elugram window—showing baseline and flow corrected data used for MWD calculations
- Mass Distribution window—showing the results in numeric and graphical form
- Calibration window—showing a graphical display of the calibration curve and the calibration table (minimized)

The three earlier windows belong together as they reflect the path of GPC data processing. Several data files (*.ch) can be loaded simultaneously and depending which Raw data window is activated the content of the other two windows will be changed to show the related Elugram and Mass Distribution. The set of windows for a data file will also update itself automatically, if the user changes parameters (for example, a change in baseline will automatically update the Elugram display and recalculate GPC results):

1. first, data are loaded from an existing file and displayed in the Raw data window. Sample information (like concentration of components, molar masses of standards, etc.) and the proper calibration curve can be entered using the associated menus. Baselines are set and an internal standard (also called flow marker) correction is performed to get reliable and reproducible data.
2. second, (in the Elugram window) integration limits can be set independently from the baseline setting to allow maximum flexibility for accurate GPC calculations. Overlays can be done here to compare GPC data

conveniently. Further options for special calculations (for example, system test with plate count and resolution calculations are located here also).

3 finally, these optimized data are used to calculate the molar mass distribution and molecular weight averages shown in the Mass Distribution window. Additional options for MWD display (cumulative or number distributions) or calculations (determination of MWD minima, maxima etc.) can be selected.

Another (independent) window can be maximized/opened from the Agilent GPC data analysis software menu using the Window Calibration command–the Calibration window combines all commands and features for full-fledged GPC molar mass calibration. Calibration files can be loaded separately into this window for review, printing and manual modification, or it is used to add calibration data points from additional data files loaded in the Agilent GPC data analysis software.

Clicking on the top bar of the individual window can activate each of the above windows. This will call up individual top menus to access the different functionality. If you like to change the scale move the mouse on the scale drag it either up and down or to the left and right. If you move the mouse into the corners on the scale you find arrows to enlarge or reduce the scale.

Preparation for GPC Data Processing

Start your Agilent ChemStation software session for the appropriate LC instrument 1 or 2 and activate the Data Analysis view. After loading a data file which you would like to process you select GPC > Activate GPC and the GPC settings dialog will be opened. If you want to create your own calibration curve see "Create a New Narrow Standard Calibration Curve" on page 38 for details, otherwise use the default calibration file (subdirectory: hpchem\gpc\calib\ def.cal) for familiarization. In the Report Settings section of the GPC Settings dialog, select Print results or interactive screen review. You might also select different colors for each signal by clicking onto the color selection box in the Detector Configuration section and picking the color from the pop-up box. For more information on the report settings see "Automated Analysis of an Unknown Sample with the New Calibration" on page 42. Press the OK button when ready to return to the Data Analysis view of the Agilent ChemStation. If you want to save the GPC settings now with the Agilent ChemStation method you click on the Save current method icon.

Start Manual Data Processing

If you want to evaluate the data loaded select Calculate GPC Results from the GPC menu in the Data Analysis view of the Agilent ChemStation. The Agilent ChemStation will start integrating the chromatogram and then will switch to the Agilent GPC data analysis software to show the GPC results in the Agilent GPC data analysis software window or initiate the report printing directly to the default printer on your PC. Switch back to the Agilent ChemStation Data Analysis screen if you want to analyze more GPC runs in the manual-processing mode.

Create a New Narrow Standard Calibration Curve

Load an existing data file in the Data Analysis view of the Agilent ChemStation—obtained with narrow standards of known molar mass. Select GPC Settings... from the GPC menu and check if the GPC settings reflect your data file and method settings (for example, detector configuration). Make sure that the Report Settings section of the GPC Settings dialog is set to Interactive Screen Review. Press the OK button when finished to return to the Data Analysis view of the Agilent ChemStation. Select Calculate GPC Results from the GPC menu to evaluate the data loaded in the Data Analysis view of the Agilent ChemStation. The Agilent ChemStation will start integrating the chromatogram and then will switch to the Agilent GPC data analysis software.

Click on the Raw data window top bar to activate and you can select Editor > Sample from the top menu. In the Sample editor selection box you can enter the sample information for this standard, for example, molecular weight information for up to 4 compounds. You may enter further information for documentation purposes.

Close this dialog box by clicking on the OK button.

In the Raw data window you may modify baselines by clicking and dragging the red triangle, which is located on the x-axis, to the position were you feel the baseline should start or end.

Select Window > Calibration from the Agilent GPC data analysis software menu. Create an empty calibration file by clicking on File > New.

Activate the Elugram window and click with the right mouse button below the (first) peak below the x-axis and select find maximum from the pop-up command box. The Add To Calibration dialog box opens to display the results for this calibration point. Check out if the elution volume of the calibration standard is correctly found and select the proper calibration molar mass of the standard from the list by clicking on the correct radio button and clicking on the add to calibration button. Continue this for all standards in this chromatogram.

Load another run with narrow calibration standards from the Data Analysis view of the Agilent ChemStation and process them in the same way until you have added all calibration standards to the calibration table. If you have completed the calibration table activate the Calibration window in the Agilent GPC data analysis software and choose a regression model from the Fit drop-down selection list in order to create a calibration curve. Save the calibration file using the File > Save As dialog from the menu and give a descriptive name for this calibration.

Quality of the Calibration Curve

The quality of the calibration curve can be controlled by:

- the percentage deviation for each calibration point as displayed in the calibration table.

  The GPC standards ISO/EN 13885 and DIN55672 require that the percentage deviation for each calibration point, given by

  $$M_{p,\text{ calibration value}} - M_{p,\text{ calculated}} / M_{p,\text{ calibration value}} \times 100$$

  shall be plotted against the elution volume. From this graph it should be possible to assess whether the positive or negative deviations are random along the elution volume axis. Calibration-curve fits which exhibit trends in the deviation plot over particular elution ranges are unsuitable. If such distributions of residuals cannot be improved upon with the regression models available in a laboratory, the results must be expected to contain greater errors and shall be stated in the test report.
- the least squares fit χ^2 which is calculated as follows:

  $$\chi^2 = \sum (\log M_{p,I} - \log M(fit)_I)$$

  with

  $M_{p,I}$ = Molecular weight of calibration standard I

  $M(fit)_I$ = Molecual weight from the calibration curve at elution ffg volume of standard I

For an ideal fit (no deviation) between all calibration points and the calibration curve the chi square value is zero.

The regression coefficient R which is calculated as follows:

$$R = \frac{cov(x,y)}{\sqrt{var(x)var(y)}} = \frac{\sum_{i=1}^{x} (xi-\bar{x})(yi-\bar{y})}{\sqrt{\frac{1}{n}\sum_{i=1}^{x}(xi-\bar{x})^2 \frac{1}{n}\sum_{1=1}^{x}(yi-\bar{y})^2}} \qquad -1 \le r \le 1$$

with:

cov(x, y)	co-variance between x and y
var(x)	variance of x
var(y)	variance of y
$\bar{x}$	mean of x
$\bar{y}$	mean of y
n	number of data points

For an ideal fit (no deviation) between all calibration points and the calibration curve regression coefficient value is 1 or -1.

Automated Analysis of an Unknown Sample with the New Calibration

Switch back to the Agilent ChemStation standard software and activate the Data Analysis view. Select GPC Settings... from the GPC menu and select the name of the previously created calibration curve from the explorer listing using the Browse button. In the Report Settings section of the GPC Settings dialog, select Print results and click on the configure print button to set up the GPC report. You can select to get additional reports on subsets of the molecular weight distribution. If you are interested in mass fractions at specific molecular masses you check the radio button and enter up to 5 different molecular masses (in Dalton). If you prefer reports on the molar masses at certain percentages of the molecular mass distribution you check the lower radio button and you can enter the percentages.

When you select to save the report as a text file the method information, the molecular weight results and slice information will be stored.

Slice information consists of: slice number, retention time, molecular mass, logarithm of the molar mass, area obtained from elugram, for example rid 1A/elu, contribution of the slice to the differential molecular weight distribution, for example rid 1A/MWD, and cumulative %, for example Cum.Dist/MWD.

You can view the content of the file in Notepad by double clicking on it. Further data evaluation/ data reduction can be performed with typical spreadsheet applications.

Press the OK button when ready to return to the Data Analysis view of the Agilent ChemStation.

Switch to the Method and Run Control view in the Agilent ChemStation and setup your system to run a single unknown or a sequence of unknowns, which shall be processed in the same way. When preparations for the run are done, start the run in the usual way. If you will be prompted to save the modified Agilent ChemStation method click on Yes to save the modified GPC settings with the Agilent ChemStation method. The automated GPC analysis is running and you should be able to collect the GPC report – and other reports as specified in the Agilent ChemStation method – from your default printer.

Comparing Existing Data Files—Overlays

From the Agilent ChemStation Data Analysis view use the Switch To GPC entry in the GPC menu to proceed to the Agilent GPC data analysis software. There you activate the Raw data window and select Raw data > Load from the top menu bar and load the Raw data file. Select the signal (*.ch) from the subdirectory (*.d). Load the appropriate calibration file by clicking on the Calibration box and selecting from the list. When the Elugram and Mass Distribution windows are updated activate the Elugram window. Select Overlay > Include curve to include the data file/signal in the overlay. You repeat this for all data files and then switch to the Overlay mode by selecting Overlay > Overlay. Now the Elugram and Mass Distribution window contain the overlaid data. Select Curves > Stacked Plot to obtain a pseudo-3D-plot.

Recalibrating an Existing Calibration Curve with Existing Data Files

Start the Agilent ChemStation software and activate the Data Analysis view. First open an existing data file from the File > Load Signal dialog, which contains calibration standards you want to use for recalibration. Locate the GPC menu and select Activate GPC, if this is not already active. In order to automate the recalibration process, it is important that you use the same standards for recalibration that you used for the original calibration. The Agilent GPC data analysis software will look for the peak position and the name of the standard to identify it for automated recalibration. Please make sure that you use the identical name for the same standard, otherwise the automated recalibration will fail.

Select GPC Settings... from the GPC menu and select the calibration file to be recalibrated by this run.

NOTE The original calibration file will be overwritten; if you want to compare the original calibration file with the recalibrated file, save it with a different name or in a different place.

Select the signal that shall be used for recalibration from the selection list for the Reference Detector for recalibration. If you have included an internal standard in your calibration standard and want to correct the elution volume click on the check box for the Reference Detector for internal standard correction and do the same selection as above. Enter the expected elution volume for the internal standard in the field for Reference Position. Change the setting (default: 5%) for the Maximum Deviation of the internal standard position to the appropriate value. Set the Report Settings according to your needs: select Interactive Screen Review for manual inspection of the recalibration process or select Print results, if you want an automatic printout of the recalibration. Press the OK button when ready to recalibrate and return to the Data Analysis view of the Agilent ChemStation. Select Recalibrate GPC Calibration Curve from the GPC menu. The Agilent ChemStation starts the integration and recalibration process and you will either receive a

recalibration report or the computer will switch to the Agilent GPC data analysis software to show the run on the screen. Continue like this if you want to recalibrate your system step by step.

Alternatively, you can recalibrate the system running a sequence of narrow standards as described below. For review the updated calibration file can be loaded in the Calibration window of the Agilent GPC data analysis software "Comparing Original and Recalibrated Calibration Curve" on page 48.

Recalibrating an Existing Calibration Curve with a New Sequence

Go to the Agilent ChemStation software and select the Data Analysis view. Make sure that the GPC option is activated in the GPC menu. Select GPC Settings... from the GPC menu and select the name of the calibration file to be recalibrated using the Browse button.

NOTE The original calibration file is overwritten. If you want to compare the original calibration file with the recalibrated file, save it with a different name or in a different folder.

Select the signal which shall be used for recalibration from the selection list for the Reference Detector for recalibration. If you have included an internal standard in your calibration standard and want to correct the elution volume click on the check box for the Reference Detector for internal standard correction and do the same selection as above. Enter the expected elution volume for the internal standard in the field for Reference Position. Change the setting (default 5%) for the Maximum Deviation of the internal standard position to the appropriate value. Set the Report Settings according to your needs: select Interactive Screen Review for manual inspection of the recalibration process or select Print results, if you want an automatic printout of the recalibration. Press the OK button when ready to recalibrate and return to the Data Analysis view of the Agilent ChemStation.

Switch to the Method and Run Control view in Agilent ChemStation and edit the Agilent ChemStation method to run a sequence with a single or a series of recalibration standards. Edit the Sequence > Sequence table and select either Calibration or Calibration Average as sample type for every recalibration standard. Enter the name of the calibration standard exactly as it was entered for the original calibration data file which is now to be recalibrated. In case of a mismatch the automated recalibration will fail. After editing the sequence table click OK and start the sequence as usually. If you will be prompted to save the modified Agilent ChemStation method click Yes to save the modified GPC settings with the Agilent ChemStation method. The automated GPC recalibration is running and you will receive a GPC recalibration report for

every recalibration sample in your sequence table or you can view the results on the Agilent GPC data analysis software windows. Be aware that the number of windows available for raw data is limited to about 10.

Comparing Original and Recalibrated Calibration Curve

The way to do this on-screen is to open the Calibration window in the Agilent GPC data analysis software. From the Agilent ChemStation Data Analysis view use the Switch To GPC entry in the GPC menu to proceed to the Agilent GPC data analysis software. Select Window > Calibration from the menu bar and load the current (recalibrated) calibration file from the File > Load dialog. Do the same with the copy of the original calibration curve used for recalibration.

NOTE This will only work if you made a copy of the calibration file before recalibrating it.

You can review both calibration files individually by selecting the proper file name from the File drop-down selection list. The contents of the graphic and table will change simultaneously without user interaction. If you would like to see an overlay of both calibration curves, click on the Overlay button on the button bar.

Using System Verification

It is a common request in regulated laboratory environments to periodically provide evidence that a software is calculating properly. Agilent GPC data analysis software features a system verification as a separate function. Go to the Data Analysis view in the Agilent ChemStation software and select GPC > system verification.

A data file and calibration file—provided as a protected part of the program - will be processed and a report will be generated as a print-out. This has to be compared to the documentation provided in this reference manual. The GPC raw data from the known sample are processed in exactly the same way as data which will be acquired by the Agilent ChemStation. This ensures that not only the final calculations are verified but also the complete data processing path.

You can find the description of the expected results in the Appendix 6, “System Verification”. The System verification is passed if results differ less than 0.5%.

4
Interactions Between Agilent ChemStation and the Agilent GPC Data Analysis Software

Interactive Data Analysis From the Agilent ChemStation Data Analysis View

The Agilent GPC data analysis software allows data evaluation of Agilent ChemStation data files using GPC data analysis functions. In the Data Analysis view of the Agilent ChemStation the GPC menu contains the commands to perform the GPC data analysis interactively. These commands are:

- Activate GPC
- Deactivate GPC
- GPC settings...
- Switch to GPC
- Calculate GPC results
- Recalibrate GPC calibration curve
- System Verification
- GPC help

Activate GPC The Agilent ChemStation loads a set of default parameter for the GPC data analysis including the calibration file (def.cal) and instructs GPC data analysis to open its GPC Settings dialog (see below). The user may set the appropriate parameters and if OK is pressed, the GPC Settings become an integral part of the current Agilent ChemStation method. The Agilent ChemStation will automatically save and load the GPC Settings together with the other parameters of the Agilent ChemStation method and will mark a change of GPC Settings as change of the Agilent ChemStation method. If you select Cancel immediately after activate GPC, the GPC data analysis will be deactivated.

While the GPC data analysis is active no other custom data analysis macro can be used with this method.

Deactivate GPC Removes the Custom Data Analysis Macro named GPC in the Runtime Check List of the Agilent ChemStation Method. Standard data analysis or any other customized data analysis can be started.

GPC Settings Opens the GPC Settings dialog box of the GPC software to enter GPC parameters, for example, the calibration file ("The Calibration Window" on page 87). These settings will be saved as part of the Agilent ChemStation method if GPC data analysis is activated. The path for the selected calibration file is included.

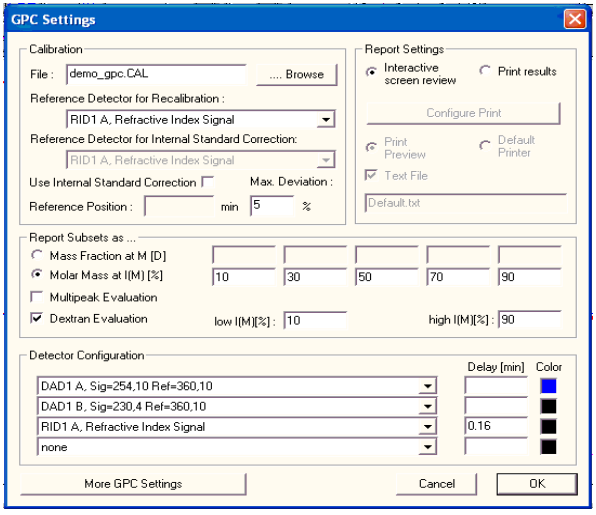


Figure 2 GPC Settings

Before editing the GPC settings an Agilent ChemStation data file has to be loaded to provide information about the GPC system parameters, for example, detector type and signal.

For any GPC data analysis it is mandatory to select a GPC calibration file. If no calibration file is available a default calibration has to be used (default.cal).

If several detectors are used the detector for recalibration and internal standard correction can be selected. You can enter delay times for additional detectors and thus use a single calibration file with up to 4 detectors in series. You can also select the colors used for display by clicking on the colored boxes. The colors will be transformed into different line styles ("Relation between Curve Color and Line Style in Monochrome Printing" on page 104).

Reports on subsets of the MW distribution ("Automated Analysis of an Unknown Sample with the New Calibration" on page 42) can be obtained for molar mass fractions or percentage of the total distribution.

When Multipeak Evaluation and Print results are selected a second results page is available either with Print Preview on the screen or with Default Printer as hard copy. It shows for up to 10 peaks integrated by the ChemStation the molecular weight results Mn, Mw, Mv, D, [n], Vp, Mp, A and A[%]. The results are calculated for the 1st signal of the Detector Configuration in GPC Settings. Page 1 is still the standard report with the molar mass results for the peaks between first start and last stop integration mark. Multipeak evaluation is only supported with "Use Integration Results" selected in More GPC Settings. This is the factory default setting.

When Dextran Evaluation and Print results are selected a second results page is available either with Print Preview on the screen or with Default Printer as hard copy. The default values for low I M [%] and high I M [%] are 10 and 90 as specified in the Pharmacopoeia. They can be changed by the operator. Dextran evaluation displays on page 2 for the 10% low-, the 10% high molecular weight fraction and the medium fraction the molecular weight results Mn, Mw, Mv, D, [n], Vp, Mp, A and A[%]. Page 1 is still the standard report with the molar mass results for the peaks between first start and last stop integration mark. Dextran evaluation is only supported with "Use Integration Results" selected in More GPC Settings. This is the factory default setting.

The results of Multipeak and Dextran Evaluation are not transferred to the text file.

The results are calculated for the 1st signal of the Detector Configuration in GPC Settings.

Multipeak and Dextran Evaluation when the data file has more than one signal:

Load all signals in the ChemStation and specify in Detector Configuration of GPC Settings that signal for which Multipeak Evaluation or Dextran Evaluation is required as the top one. Multipeak evaluation and Dextran Evaluation will be performed for this signal.

Multipeak- and Dextran Evaluation is not supported for data files with more than 4 signals.

The identification and integration of the peaks of interest can be performed in two ways, which are selectable in the More GPC Settings window:

1 with the ChemStation integrator

2 with Fixed settings

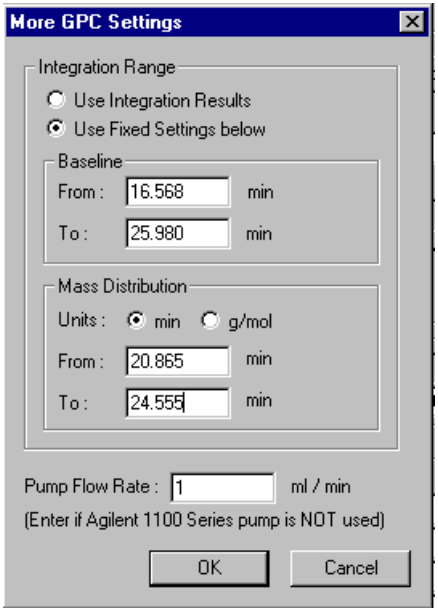


Figure 3 More GPC Settings

By default Use Integration Results is selected and the GPC data analysis software uses the results of the ChemStation integrator. You can change to Use Fixed Settings below. You have to specify then from and where to the baseline shall be drawn and the evaluation range for the mass distribution window (refer also to "Optimization of the Integration Parameters" on page 57). If an Internal Standard Correction is in use the times are updated.

The Agilent ChemStation GPC data analysis software transforms the peak retention times into elution volumes for the calculation of the molecular weight averages and the mass distribution. For this the software automatically retrieves from the Agilent 1100/1200 Series pump the flow rate. If a 1100/1200 Series pump is not used specify the flow rate in the Default Flow Rate box. If automated data analyses is desired this should be done before the data acquisition is started. The default flow rate after installation is 1 ml/min. If such a configuration is used the flow rate can still be changed after data acquisition. If an Agilent 1100/1200 Series pump is used the flow rate is permanently stored as part of the data file.

Switch to GPC

Opens the Agilent GPC data analysis software window that is used to review GPC data interactively and set up calibration curves.

Calculate GPC results

The command Calculate GPC results will initiate

- the integration of the chromatogram by the Agilent ChemStation based on the selected integration parameters,
- the transfer of raw data to the GPC Raw data window,
- the compilation of an interface file, and
- the calculation and display/report of GPC results according to GPC settings.

The standard Agilent ChemStation data analysis software is used to identify the peaks of interest for the GPC calculations. The integration parameters should be optimized in order to include all peaks of interest.

For the currently loaded data file the Agilent ChemStation generates an interface file (GPCintf.txt) which holds additional sample information about the data file. Selecting Calculate GPC Results opens the Agilent GPC data analysis software and evaluates the current data file according to the information of the interface file. The interface file holds information about the Agilent ChemStation method, GPC settings, file info, acquisition parameter, column information, signal information and integration results.

This command is also used to load the data file into the interactive GPC Raw data window when you like to set up a new calibration. It is recommended that the default calibration file gpc\calib\default.cal is loaded. It is required that the Report Settings section of the GPC Settings dialog are set to Interactive Screen Review when you want to set up a new calibration curve.

Recalibrate GPC Calibration Curve

This command works the same way as Calculate GPC Results but the Sample type in the interface file is set to Calibration. The GPC data analysis software performs an update of the calibration table specified by the GPC settings of the Agilent ChemStation method. In the dialog box you can choose to either average or replace the old calibration points with the new ones.

See “Automated Data Analysis” on page 60 for doing automated recalibration as part of a sequence of samples.

System Verification

Starts the System Verification of the GPC data analysis software. It takes a set of raw data and a predefined set of GPC parameters to obtain GPC results. These results can be compared to a set of GPC results for these raw data documented in the Chapter 6, “Appendix”. A failure to obtain results within 0.5% deviation indicates a software failure. Please contact Agilent.

GPC-Help

Starts Help of the Agilent GPC data analysis software.

Optimization of the Integration Parameters

The identification and integration of the peaks of interest can be performed in two ways:

- with the ChemStation integrator
- with fixed setting

With the ChemStation Integrator

By default the GPC data analysis software uses the integrator. You can change to Use Fixed Settings below in the More GPC Settings window which you obtain from the GPC Settings screen (see "Interactive Data Analysis From the Agilent ChemStation Data Analysis View" on page 52).

The standard Agilent ChemStation data analysis software is used to identify the peaks of interest for the GPC calculations. Optimize the integration parameters in order to include all peaks of interest according to whether it is a narrow standard, a broad standard or a broad sample. It is recommended to use the Agilent ChemStation enhanced integrator. The baseline will be drawn straight from the start to the end of integration in the Agilent GPC data analysis software.

Narrow Calibration Standards

Narrow calibration standards are injected either as single compounds or as mixtures, typically 3 to 5 standards with a wide difference in the molecular weight to ensure separation. They should be analyzed as unknown samples to set up a calibration curve. Data files are loaded in the Agilent ChemStation Data Analysis and integrated.

In both cases the Agilent ChemStation integration parameters should be selected that all peaks of interest are integrated, excluding monomeric compounds and internal standards.

The most important parameters are:

- slope sensitivity
- peak width
- area and height reject

The correct integration (peak area) is not important since only the elution volume is of interest and will be determined automatically in the Agilent GPC data analysis software.

Broad Standards and Samples

Broad standard and sample chromatograms in GPC are characterized by broad peaks (peak width at half height typically between 0.5 and several minutes) with flat slopes. For the GPC evaluation of such peaks it is important that start and stop marks are determined correctly.

Correct drawing of baseline in the Agilent ChemStation standard data analysis has no influence. The Agilent GPC data analysis software always draws the baseline exactly from the first start to the last stop mark.

The following integration events are available in the Data Analysis view of the Agilent ChemStation for optimization:

- Choose a low slope sensitivity, for example, 0.01, to ensure that the start/stop marks are set as close as possible to the peak start/end.
- Choose peak width close to real peak width at half height.
- Use Integration Off and Integration On to transfer only the polymer peak to the GPC data analysis software. If a flow marker is used there is no need for integration of the peak. The GPC data analysis software will search in a reference window as selected in the GPC settings.
- Choose area and height reject to exclude minor peaks.

Refer to the Help topics on further available integration events in the Agilent ChemStation.

With Fixed Settings

Using Fixed Settings means that you specify time controlled from and to where the baseline is drawn as well as the range of the mass distribution. For the fixed settings of the mass distribution a g/mol unit instead of minutes can be selected.

The Fixed Settings possibility is recommended for QA/QC type analyses of similar polymers or when the integration with the enhanced integrator does not produce satisfactory results.

Automated Data Analysis

The link between the standard Agilent ChemStation and Agilent GPC data analysis software is done via the Agilent ChemStation concept of a Custom Data Analysis Macro in the Runtime Check List of the Agilent ChemStation method. Once GPC Data Analysis is activated via the menu Activate GPC a macro named GPC is entered as Custom Data Analysis macro.

This GPC macro is always executed if the user performs a Run Method or Run Sequence and has activated the Agilent GPC data analysis software. It performs automatically the same operations as described for the interactively used Calculate GPC Results and Recalibrate GPC Calibration Curve from the GPC menu. If the user does a single run via Run Method then the raw data are processed as sample data by the Agilent GPC data analysis software. If a sequence of samples is analyzed or reprocessed the field is the sample type, that can be either Sample or Calibration. See the details on the Sequence > Sequence Table in the standard Agilent ChemStation Method and Run Control. In the case that the sample type is set to Sample the GPC data analysis will calculate and report the molecular weights according to the GPC Settings. If the sample type is set to Calibration the data file will be used to recalibrate the system by overwriting the calibration file specified in the GPC settings of the Agilent ChemStation method.

It is possible to use the standard Agilent ChemStation Data Analysis functions in parallel to the GPC data analysis by checking the appropriate fields in the Runtime Checklist. With the appropriate Report Settings in the Agilent ChemStation and Agilent GPC data analysis software you can generate a Agilent ChemStation report (except a calibrated report) and GPC report for each run. You can also do a reprocessing sequence for previously measured data as you do within the standard Agilent ChemStation data analysis.

NOTE

The following fields in the Sequence Table are interpreted by the Agilent GPC data analysis software: Vial, Sample Name, Method Name, Inj/Vial, Sample Type, Cal Level, Update RT, Interval, Data File, Inj Volume.
The GPC data analysis software ignores the following fields in the Sequence Table: Update RF, Sample Amount, ISTD Amount, Multiplier, Dilution, The Sample Type Control is not supported. It is treated like a Sample. In this case a warning entry is made in the Sequence Logbook of the Agilent ChemStation.

Automated GPC Recalibration

Calibration in GPC typically needs several calibration standards to generate a new GPC calibration table or fully recalibrate an existing GPC calibration table. Recalibration in a sequence will be setup with several consecutive sequence lines specifying calibration standards using one or more appropriate methods. The Sample Type needs to be set to Calibration in the sequence table to perform a re-calibration with the acquired data file.

The type of recalibration is depending on the setting in the column Update RT of the Agilent ChemStation Sequence Table.

If Update RT is set to Replace then the value of the expected elution volume in the GPC calibration table will be replaced by the new calculated elution volume.

If Update RT is set to Average then the new calculated value of the elution volume in the GPC calibration table and all measured elution volumes since the last Replace Re-calibration will be averaged and this average will be entered into the GPC calibration table.

If Update RT is set to NoUpdate then this sample will be treated as an unknown sample. No changes are made to the GPC calibration table. A warning entry is made to the Sequence logbook of the Agilent ChemStation.

No recalibration will occur if the peaks are found outside the specified reference window.

NOTE

If the user has selected Cyclic Recalibration in the Agilent ChemStation by entering a number in the Interval column of the sequence table he must enter the value 1 for the Cal Level for each calibration line in the sequence table, because all standards are of the same concentration. The values for Interval must be the same for a group of consecutive calibration lines

5
Software Windows and Menus

Description of Menus

Menu items in the Chromatogram window:

Menu items in the Elugram window:

Menu items in the Mass distribution window:

Menu items in the Calibration window:

A toolbar will always take up the section below the menu bar. The buttons always look the same, but can perform different tasks depending on the active subwindow, for example, the File Open button loads raw data files when pressed with the Raw data window being active, but will open calibration files while the Calibration window is on top.

The status area is located below the toolbar.

This section contains information about the currently loaded data file in the interactive screen of the Agilent GPC data analysis software. It shows the name of the active detector signal (detector selection field) on the left side and the assigned color of the corresponding curve (detector color selector field). If several detector signals are loaded the active detector signal in comparison to others is distinguished by the fact that any scaling-, color giving and search functions relate to it. To change the active signal click on the detector selection field of the active curve with the left mouse button. To change the color of the active curve click on the detector color field, and choose the color you wish from the color selector panel. Next to this you can read off the x-coordinate of the cursor and the y-coordinate of the active curve.

In the central section of the status area the name (field Sample) of the sample which is presently loaded in the active Raw data window, as well as the currently used calibration curve are displayed. Upon clicking the field Sample you can change to any loaded (but hidden) Raw data window or search for samples. If you select Search sample a dialog box allows you to select search criteria and start a Search. If you like to load any of the listed data files highlight the name and select Open. The Browse button lets you define a disk drive and directory where to search for raw data files.

The View selection field allows to store 4 different views of the windows for Raw data, Elugram and Mass distribution. The windows can be arranged on the screen by the user and will be stored when another View is selected. The window arrangements can be rearranged by hitting the functional keys F5-F8 or by selecting from the View selection field.

The Window Menu

The Window menu is available with any of the context sensitive menus. It allows to change to other subwindows or changes the arrangement of the windows.

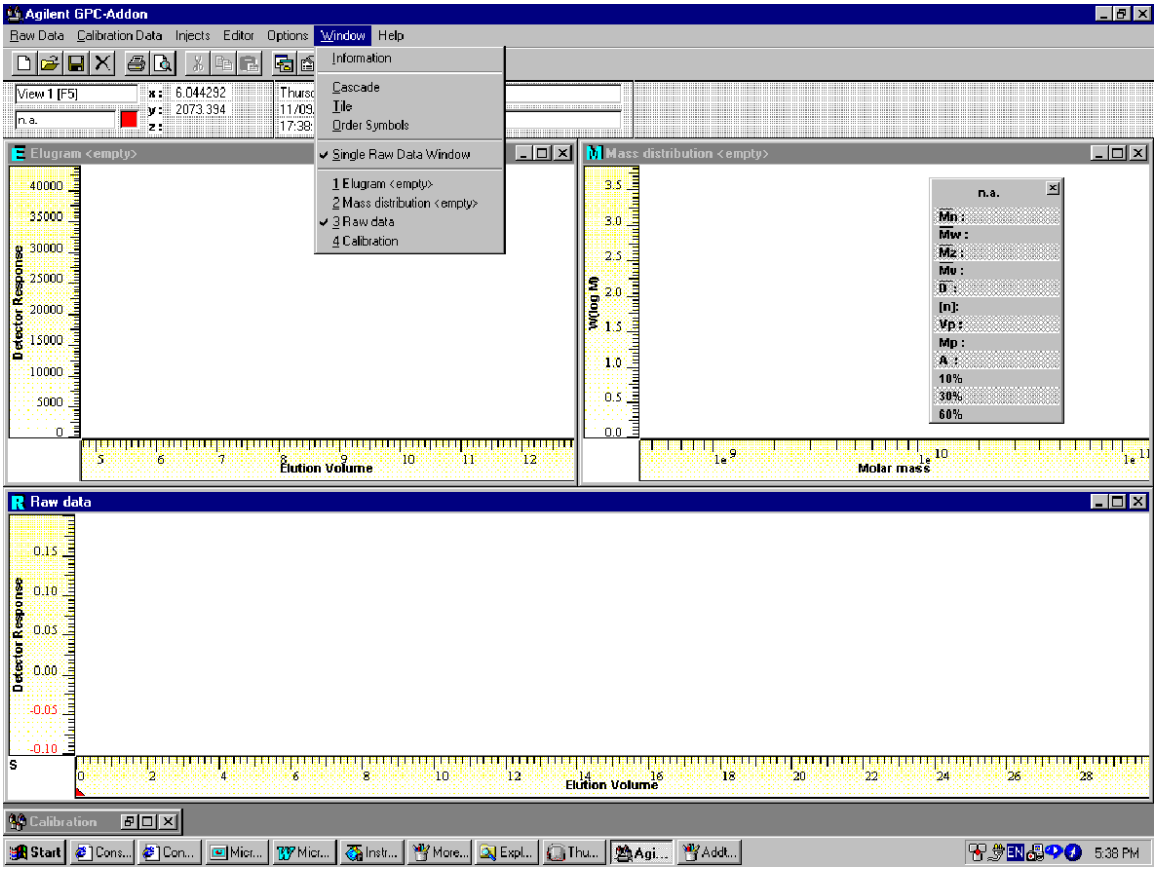


Figure 4 Window Menu

Window Information Inserts the Information window in the Raw data window, in the event that this has been deleted previously by double clicking on the window.

Tile The standard setup arranges the windows according to a scheme given by the system. A useful representation for the evaluation of the collected data can be created, if only one Raw data window with the respective Elugram and Mass distribution window are open. After selecting Window > Tile the window representation now will be selected in such a way that the Raw data window in the lower section of the display is displayed wide, while elugram and mass distribution share the upper portion of the display. With this you will obtain direct control during the evaluation how a correction in one window directly influences the results shown in the upper windows. By selecting Window>Tile the standard setup is typically obtained when the system was before in another setup.

Single Raw data window With two instruments connected or multiple data sets loaded - and switched-on Single Raw Data Window mode (recognizable by the hook) only one set of raw data will be displayed, non-active data will be filed as symbol <date>. By switching-off the Single Raw Data Window mode several Raw data windows can be displayed at the same time.

Raw Data Window

The Raw data window displays the chromatogram of data loaded from the file system. If the data file has been processed before already by the Agilent GPC data analysis software the processing parameters are loaded, e.g. the calibration file. For each data file loaded a Raw data window will be created. Approximately 10 windows can be created. If the raw data file is loaded without prior processing default parameters (see Appendix 6, “Appendix”) are used, for example, for flow rate and internal standard reference position.

Window Description and Options

The axes that are assigned to the various detector signals are located on the left edge of the window. Because the Agilent GPC data analysis software processes several detectors simultaneously, you determine by selection of the active curve, which curve pertains to scaling operations, peak searches and similar. The active curve can be selected by high lighting the corresponding detector in the moveable information window or alternatively in the status area.

Colored triangles can be seen underneath the x-axis. Red triangles define start- and end position of the baseline (only visible after defining the baseline), green triangles define the position of the internal standard. If no baseline has been defined for a data file the red triangles are partially hidden in the left corner. The mark for the internal standard is dark green, if the value of the calibration curve is defined for the measurement. After the internal standard of measurement has been defined, by either setting or searching the internal standard, it will be marked in light green color.

Table 3 Markers Under the X-axis

Color of Triangle	Meaning
Red	Baseline marker
Dark green	Location of the internal standard (not used)
Light green	Location of the internal standard (used)

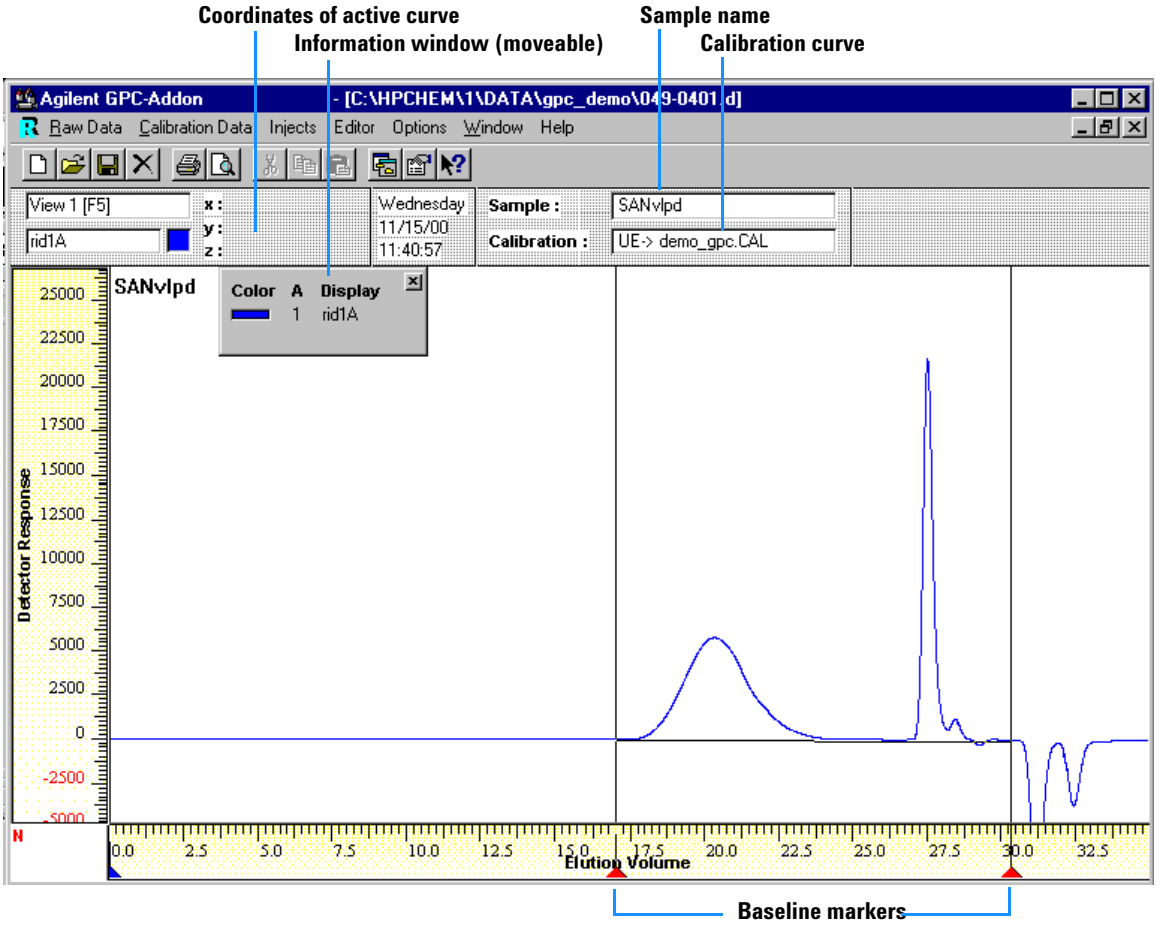


Figure 5 Raw Data Window

Windows can be zoomed (magnifier-effect) for a better recognition of certain window sections. To do so click into the window pressing the left mouse button, keep the button pressed and pull the appearing rectangle until it encloses the section which is to be magnified. After releasing the left mouse button the magnified section is displayed. To unzoom the selected region click the right mouse button into the window.

Manual Baseline setting Click onto the baseline mark and keep the left mouse button pressed. Move the baseline marker to the desired location and release the mouse button. Upon release of the mouse button the baseline marker will be set at the x-position where you release the button. Define the other baseline mark analogous corrections of the baseline can be carried out by clicking the corresponding baseline marker and relocation.

Functions of the X-axis

These functions will be accessible if you click on the x-axis scale with the right mouse button. A window appears in which you can select out of the following functions:

Internal standard cancel Deletes the value for the internal standard of the sample viewed.

Internal standard set Opens a window in which the value for the internal standard of the sample can be registered (By deviations more than 20% from the position specified in the GPC or calibration settings you will get an error message. The program assumes that the chromatographic system has changed to much to be compensated by the internal standard). The elution volume is the x-value where you have clicked to enter the function. After setting the internal standard the marker changes from dark to light green. The reference position entered here will be used as long as no new information is entered, for example, by using Calculate GPC results from the Agilent ChemStation Data analysis view.

Int. standard search maximum / minimum Searches the next maximum/minimum in the active curve and sets the internal standard (By deviations more than 20% from the position specified in the calibration file you will get an error message the program assumes that the chromatographic system has changed too much to be compensated by the internal standard). The search is started from the cursor position relative to the volume/time axis to the right side. After setting the internal standard the marker changes from dark to light green.

After the minimum/maximum is found an information window opens, where you can compare the value found with the value given in the calibration file.

Manual scaling Allows the manual setting of the window section.

Standard scaling Restores the scaling of the last manual scaling (for example, after editing the scaling by the arrow keys or the scroll bar of the x-axis.)

Properties In this dialog box you can modify the x-axis properties like background color, caption and caption style, etc.

Menu Raw Data

Load Opens the file selector dialog to load Agilent ChemStation files for interactive data processing.

Printer Setup Allows the adjustment of parameters of the active printer. However, the default printer must be defined in the Windows System control.

Landscape format prints the window contents in a page filling format. Portrait format prints beside the graphically representations also information as used detectors or molecular weight averages. The exact information of the portrait format print depends on which window will be printed.

For color printers you can switch between color and monochrome printing. The representation of the curves in monochrome-print depends on the adjusted color of curves. The correlation between curve color and line style in monochrome-print is listed in Table 4 on page 104.

Print Prints the contents of the Graphic window. Only the visible section of the window will be printed out.

Page Preview Shows a print preview and at the same time copies the contents to the Windows clipboard. Upon printing portrait format, the graphic and information for the measurements will be typed out, by landscape format printing only the graphic.

Print Options Allows the adjustment of font size on the print out. It is recommended to use 10 pts as font size for printouts. Select 12 pts for font size if you like to read the details in the page preview on the screen.

Menu Calibration Data

Load Loads a calibration file (*.CAL) into the program. The last loaded calibration file is saved together with the baseline and integration limits in a separate file (*.fst) when closing the data file. Recalculation starting from the Agilent ChemStation will overwrite existing evaluation limits stored in this file.

Information Opens a window in which the name of the calibration curve, elution volume of first and last point of the calibration curve, the value for internal standard of the calibration curve and the sample under investigation as well as the MH-Parameters of the calibration curve (default settings: A = 1.000 and K = 0.000 ml/g), will be shown. The default MH-parameters are ignored in narrow standard calculations.

Menu Editor

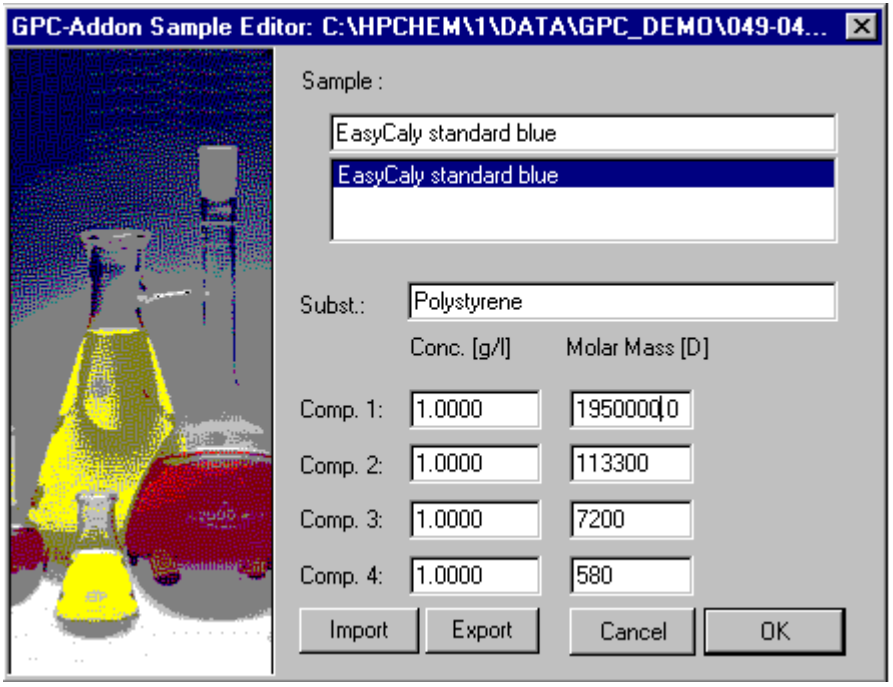


Figure 6 Sample Editor

Editor Samples Allows to add further informations like molecular weights for calibration etc. regarding the sample. The sample name can be edited here. The Editor menu is designed such that up to 4 different components can be entered (for example, for calibration mixtures). For each component its concentration and molecular weight can be entered.

After the sample name has been entered, leave the sample editor with OK or via the Return key. The entered values will then be accepted. Cancel is leaving the sample editor without acceptance of the processed changes.

The options Import and Export permit to save the input mask of a sample (Export) and to reload it at a later time (Import) if the same sample will be reanalyzed. Thereby you avoid to fill out a sample mask each time for samples being reanalyzed often, for example, calibration or reference samples.

Menu Options

Analysis contains 2 items – positive and negative peaks.

Positive / Negative Peaks Determines which peaks will be included in the evaluation. Evaluation of negative peaks (with hook on) will result in mirroring these for representation in the Elugram window. Thus, negative will appear as positive peaks in the elugram.

Color Scheme Allows the selection of window background color. Palette allows to activate a self defined color background. The creation and selection of the color background is done in the Elugram window by selecting Options > Palette. It is also possible to load a bitmap as background (bitmaps and colored backgrounds are not being considered during printing).

Grids Allows the addition of grid lines into the window.

Lines Definition of line thickness of the curves.

NOTE When closing a Raw data window the evaluation limits etc. are being saved. If you want to evaluate a sample again without overwriting the already existing evaluation limits, this can then be done by multiple loading of the same data file. One copy remains unprocessed (here you can possibly rename the baseline data to prevent confusion) while working on the other copy. It is important that when closing the data file the Raw data window of the unprocessed copy will be closed last.

Elugram Window

In the Elugram window the baseline corrected raw data are presented. The volume axis has been corrected by the internal standard if an internal standard is used. Because the volume axis is corrected for the internal standard, the positions of peak maxima in the Raw data window and in the Elugram window need not to be identical. The presented volume range corresponds to the range between the baseline markers of the Raw data window.

In the Elugram window the integration limits for calculation of the molecular weight distribution and molecular weight averages have to be set. Furthermore different injections can be overlaid in the Elugram window. The corresponding MWD curves can also be viewed as overlay in the Mass distribution window. The overlay mode is indicated by the word overlay in the headline. In addition the transfer of peak volumes and molecular weights to the Calibration window is done from the activated Elugram window.

Window Description and Options

In the Elugram window only one y-axis exists. The data are presented with its real value, or in a normalized representation by which the maximum of each curve will be set to 100%. Change over by clicking with the right mouse button on the y-axis.

To set the integration limits, click with the left mouse button on the red markers on the right and left window edge and pull these while keeping the mouse button pressed. Release the mouse button at the required positions in the Elugram. The influence to the molecular weight distribution becomes visible in the Mass distribution window. Alternative the integration limits can be entered numerically using the functions of the x-axis.

Functions of the X-axis

These functions will become accessible upon clicking on the x-axis labeling with the right mouse button.

Find Maximum Searches the next maximum in the active curve from the position where this function was called. The program opens a dialog window and adds the volume at peak maximum to the corresponding field of a dialog box. The molecular weight of the component and the sample name can be entered or selected from the selection of molecular weights given in the sample editor. Note that in order to add the data to a calibration the Calibration window has to be opened, and a new calibration has to be created using the File > New command or an existing calibration has to be loaded to the Calibration window, in order to extend this calibration.

The search routine starts at the x-position where you have entered the functions of the X-axis window, and moves upward to the next maximum.

Find Minimum Searches the next minimum in the active curve from the position where this function was called. The program opens a dialog window and adds the volume at peak minimum to the corresponding field. The molecular weight of the component and the sample name can be entered or selected from the selection of molecular weights given in the sample editor. Note that in order to add the data to a calibration the Calibration window has to be opened, and a new calibration has to be created using the File > New command, or an existing calibration has to be loaded to the Calibration window, in order to extend this calibration.

The search routine starts at the x-position where you have entered the functions of the X-axis window, and moves downwards to the next minimum.

Set peak integration Performs a peak searching routine in the active curve. The integration limits are placed around the found peak, whereby the first local minimum to the left or right of the position, from which the function have been called, will be used. The mass distribution is then calculated for only this peak.

Manual Scaling Allows to enter numeric values to select the presented volume range.

Manual Borders Allows to set the integration limits manually in form of predefined molecular weights or elution volumes.

Standard Scaling Restores the scaling settings of the last manual scaling (for example, after changing the scaling via the arrow keys or the scroll bar of the x-axis).

Menu File

Printer Setup Allows the adjustment of parameters of the active printer. However, the active printer must be defined in the Windows System control.

Landscape format prints the window contents in a page filling format. Portrait format prints beside the graphically representations also information as used detectors or molecular weight averages. The exact information of the portrait format print depends on which window will be printed.

For color printers you can switch between color- and monochrome-printing. The representation of the curves in monochrome-print depends on the adjusted color of curves. The correlation between curve color and line style in monochrome-print is listed in Table 4 on page 104.

Print Prints out the content of the Graphic window. Always the visible section of the window will be printed out.

Printer Annotation Allows the annotation of report with an additional line.

Preview Shows a print preview and transfers the contents to the windows clipboard. With portrait format printing the graphic and information to the data will be displayed, landscape format shows only the graphic information.

Configuration If the Calibration window is activated this menu item gives access to an additional menu. Here you can select to save and load configuration details for the calibration files loaded such as color of line and tags. Select Default to view and edit the details.

Menu Overlay

The menu Overlay allows to switch between a normal mode—to view single data files—and the overlay mode to overlay data files (and respective mass distributions.

Include Curve This command copies the curves presented within the integration limits into a specific memory section to view and evaluate the curve in the overlay mode (“Raw Data Window” on page 68).

Overlay Changes between normal and overlay mode (recognizable by the hook or in the status line of the Elugram and Mass distribution window). The selection of another sample by the sample selection box of the status area switches from overlay mode into the normal mode. In the overlay mode several samples can be compared visually with each other or calculation operations can be processed with the curves.

Delete all curves Deletes any curve in the overlay memory and automatically switches back to the normal mode for the Elugram window.

Save As Saves the window content as overlay file (*.ADD). This file can be loaded later e.g. for reproducibility tests by overlay, or to chronologically trace the development of separation efficiency. Overlays are also needed to start broad or integral calibration.

Load Loading a saved overlay file and switching to the overlay mode. Now further curves from Agilent GPC data analysis software can be added to the overlay (see Overlay > Include Curve in the Elugram window). Please note, that before loading of an overlay, the file must be available for the overlay, this means you cannot add an overlay file to an overlaid curve, but only curves can be added to an overlay file.

Information Opens an Information window, which displays the number of transferred samples in the overlay memory and the total number of the curves.

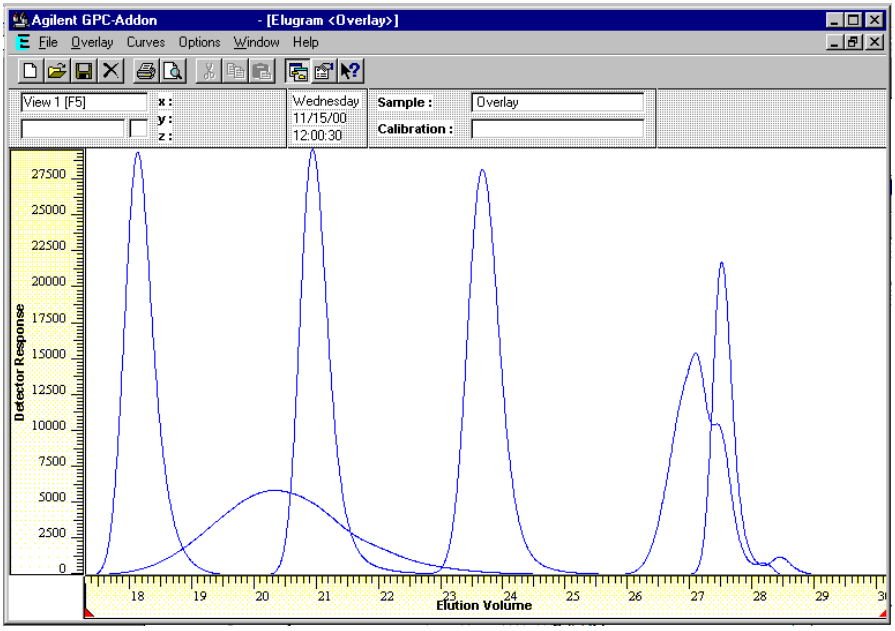


Figure 7 Elugram of Two Overlaid Curves

Menu Curves

Calibration Curve Displays the calibration curve in the Elugram window beside the elugram. Red dots mark the first and last calibration point. Now it is easy to check whether the presented sample elutes completely in the calibrated section or partially outside the calibrated section.

Curve A, B Opens a window which allows smoothing, interpolation or specific adjustment routines.

The option Interpolation defines how two consecutive points will be connected to each other. Routinely the data points are connected linear with each other. Spline however permits curved connections.

Smoothing defines smoothing routines for example, to remove spikes. For this you use adjacent averaging of neighboring points. A larger number of points increases the smoothing effect and thereby increases changes in the peaks.

The special fit Fourier transformation opens a window in which the Fourier coefficients, which are necessary for the description of the curves, will be presented. The number of harmonics define the number of terms used for the synthesis of the curve. The smaller the number of used coefficients, the higher the smoothing effect, but at the same time the general form of the synthetic curve will be more different from the original one. Upon clicking on Synthesis the calculated (red) and measured curve (blue) is presented.

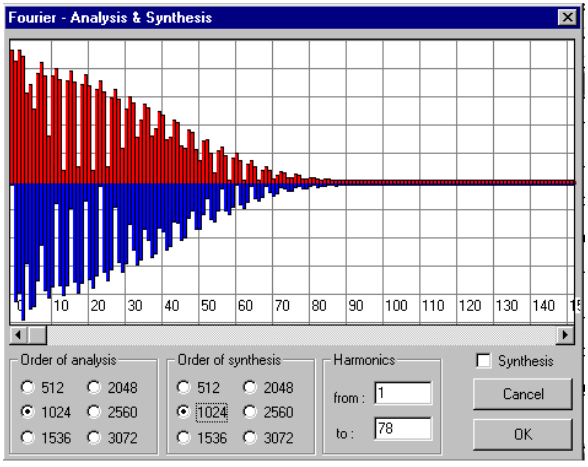


Figure 8 Fourier Analysis Window

The special fit Despike uses a special smoothing routine that removes short time detector noise (Spikes). The despike routine defines 3 data point sections. A data point section in which the viewed data is located and two further data sections to its left or right side. Each section will be averaged separately. The mean value of both outside sections will be connected by a straight-line. If the mean value of the inner section deviates by more than a given tolerance from the connecting line of the outside section, this point will be identified as a spike and ignored. The three parameters which have to be entered are therefore Width 1, the number of data points of the outside section, Width 2 the number of data points of the center section and the Tolerance.

Menu Options

Component Allows the selection of the component from the sample editor, for example, to assign a concentration or a molecular weight to a peak (see Editor > Sample). This selection is important for the creation of calibration curves.

System Test Performs a system test according to the requirements of DIN 55 672, ISO and EN13885 for the peak to be evaluated. With several peaks in the Elugram window, you can choose a single peak by selection of the integration limits. The system test calculates the theoretical plate count, the asymmetry and the separation efficiency. See "Separation Efficiency, Resolution and Plate Count" on page 31 for definitions. For the calculation a calibration curve is necessary, so that the interested peak can be pictured completely in the Mass distribution window. Also the dimensions of columns must to be known. If they are entered into the method incorrectly, the system check can be calculated with different values. This does not influence the values stored in the method.

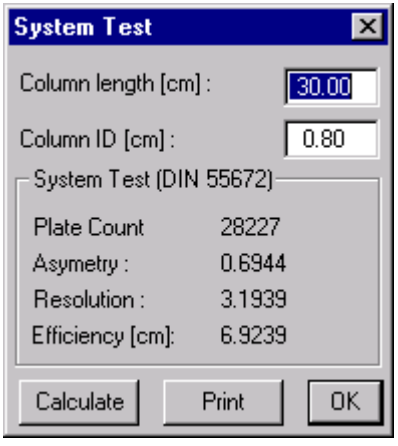


Figure 9 System Test

Stacked Plot Pseudo 3-dimensional representation. The curves will be drawn at consecutive slanted shift other to generate a spatial representation. This type of representation is also available in the overlay mode.

Overlay Mode

The overlay mode allows to overlay curves of different samples. The transfer of curves into the overlay mode happens via the Elugram window (Overlay). For viewing an overlay the menu item Overlay of the Overlay menu must be activated. This can be recognized by the hook on the Menu item Overlay or by the name of the Elugram- or Mass distribution window Elugram Overlay or Mass Distribution Overlay or by the sample name Overlay in the sample selection box of the status area. In the overlay mode the Mass distribution window also presents the results of the overlay.

The menu items of the Overlay window are not different from those in the Elugram window, merely the menu Curves has a slightly different meaning.

Upon clicking on a curve name you receive a menu, in which similar to the curve menu of the Elugram window smoothing, fit and interpolation routines or the curve color can be selected. The menu item Trace permits to see/hide the curve. Within the field Comment a comment can be entered which appears in the print out of the overlay. For the evaluation of the curve any calibration file can be entered.

Mass Distribution Window

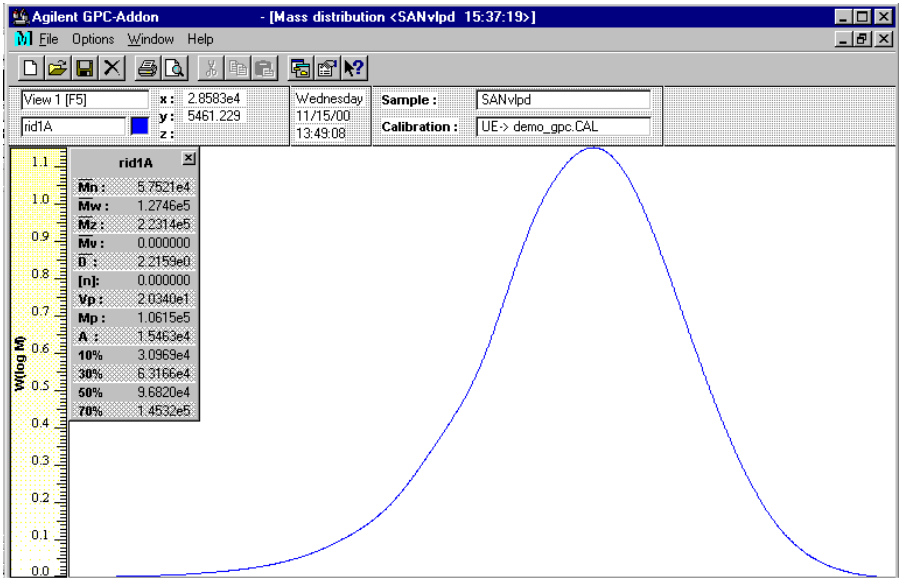


Figure 10 Mass Distribution Window

The Mass distribution window shows the molecular weight distribution of the sample. The calculated molecular weight averages and other information are displayed in an Information window. The molecular weight averages always relate to the sample within the integration limits- determined in the elugram. If no changes are made they are identical to start and stop of the baseline. The values given in the Information window are:

M_n Number average molecular weight

M_w Weight average molecular weight

M_z Z-average molecular weight

M_v Viscosity average molecular weight

D Polydispersity M_w/M_n

[η] intrinsic viscosity calc. using Mark-Houwink coefficient of calibration curve (the value is 0.000000 as long as the Mark-Houwink coefficients a and K for the calibration standards are not specified. The coefficient can be specified when you go to Calibration, then Settings. The default values are for a = 1 and K = 0.)

V_p Volume at the peak maximum of the elugram

M_p Molecular weight at peak maximum of the elugram

A Peak area

< Mass fraction with molecular weight M < M (left MWD Limit)

w% Mass fraction between the limits

> Mass fraction with molecular weight M > M (right MWD Limit)

Herein the left and right MWD limit is defined by the red triangles in the Mass distribution window which are located on the outer left and right window edge when entering the Mass distribution window. These limits have no effect on the molecular weight averages. They can be dragged by moving the cursor on the red triangles and pressing the left mouse button.

To display the mass fraction limits in the report window highlight the mass distribution window, then select **Options**, then **Fixed cum%...** and then deselect **Display numeric...**

You can also specify exact numbers when you right click at the x-axis, then select **Manual Borders** and then specify the numbers.

The Information window can be expanded to the right and at the bottom to show more results, for example, if two signals are processed. This may require to enlarge the Mass distribution window as well.

File

Printer Setup Allows the adjustment of parameters of the active printer (default printer). However, the default printer must be defined in the Windows System control.

Landscape format prints the window contents in a page filling format. Portrait format prints beside the graphically representations also information as used detectors or molecular weight averages. The exact information of the portrait format print depends on which window will be printed.

For color printers you can switch between color- and monochrome-printing. The representation of the curves in monochrome-print depends on the adjusted color of curves. The correlation between curve color and line style in monochrome-print is listed in Table 4 on page 104.

Print Prints the contents of the Graphic window. Always the visible section of the window will be printed out.

Printer Annotation Allows the annotation of the report with an additional line.

Page Preview Shows a print preview and transfers the contents to the Windows clipboard. With portrait format print the graphic and numeric results to the data will be displayed, landscape format shows only the graphic information.

Options

Cumulative distribution Activates/deactivates the representation of the integral (cumulative) distribution for all curves. The cumulative distribution is overlaid to the differential distribution.

Number Distribution Converts from mass to number distribution.

Maxima Marks the maxima of the curves.

Minima Marks the minima of the curves.

Points of inflection Marks the points of inflection.

Fixed Cum%: Edit Allows to edit a list to report subsets of the MW distribution. The molecular weights are given at which the cum. MWD reaches the predefined percentage values. MWD integrates by ascending molecular weights, Elugram integrates in the elugram (descending molecular weights).

Fixed Cum%: Display Displays the fixed cum% list in the Information window.

The Calibration Window

The Calibration window allows creating and viewing calibration files for the Agilent GPC data analysis software. It allows for the creation of conventional calibration curves by use of narrow distributed polymer standards and the transformation of calibration curves to universal calibration curves by use of the Mark-Houwink coefficients. Calibration is also possible by broad standard calibration and calibration using the integral molecular weight distribution.

Several calibration curves can be loaded for review, printed and overlaid for comparison. The name of the displayed calibration file is shown in the File selection box. Editing and reviewing in the Calibration window can be done without interfering with the automated processing or interactive screen review of data files–except when calibration files in use are overwritten. Calibration points of different calibration curves can be easily mixed by copy/paste. Individual data points can be removed in the calibration table. In calibration routines that are not often used by most customers, i.e. broad standard calibration, and calibration using the integral molecular weight distribution, the user is guided through the calibration procedure.

General Description

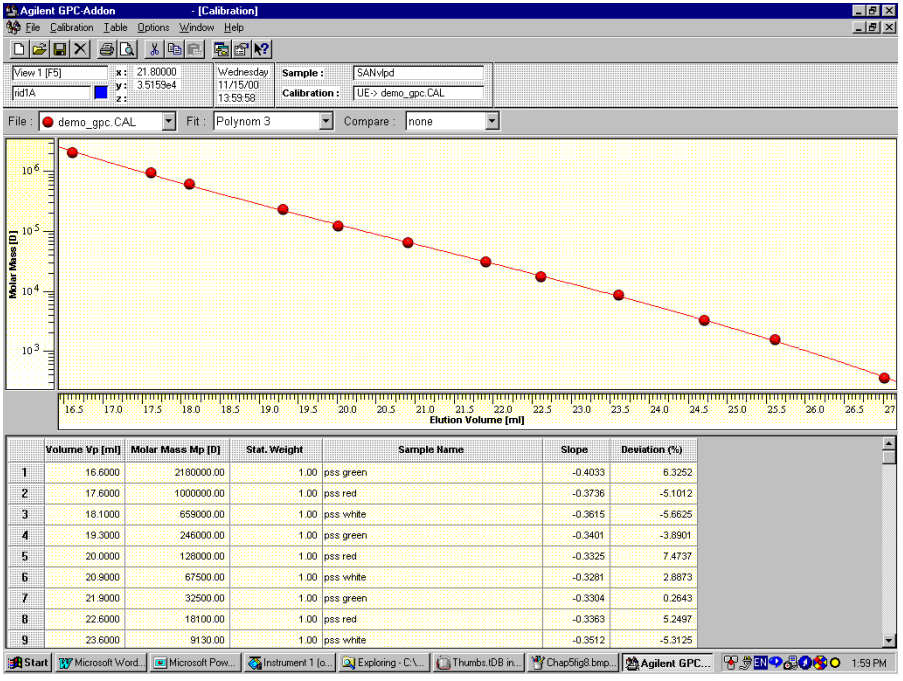


Figure 11 Calibration Window

The Calibration window itself is divided horizontally. The upper part shows the graphical information, while in the lower part the Calibration windows data editor section is shown. The relative sizes of the parts can be easily adjusted by moving the section separator.

Calibration curves can be overlaid for comparison using the overlay button.

Within the editor part (calibration table) of the Calibration window you will always see the data of the active calibration, which can be selected from the File pull down menu.

Menu Structure of Calibration Window

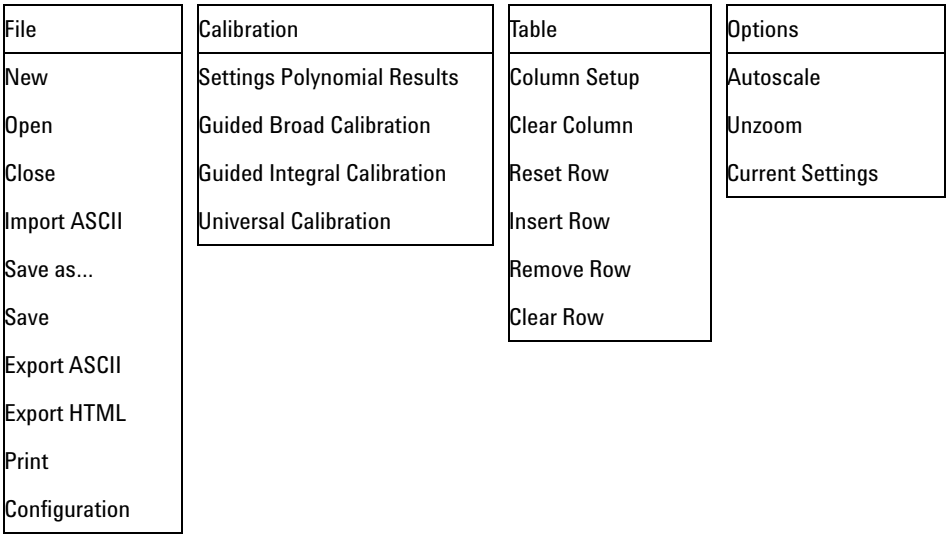


Figure 12 Menu Structure of Calibration Window

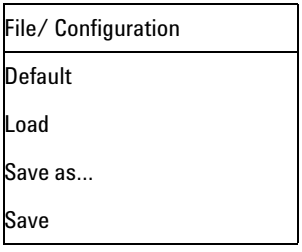


Figure 13 Submenu for File/Configuration

The File menu contains all input/output options, as well as the global view settings of the program.

New Creates an empty calibration table, automatically makes this file the active calibration file.

Open Loads an existing calibration, automatically makes the opened file the active file of the Calibration window.

Close Closes the actual calibration file.

Import ASCII Imports a data table containing elution volume, molecular weight, Sample name, automatically opens a new calibration and makes this calibration the active calibration.

Save As... Saves the active calibration.

Save Saves the active calibration under its actual name.

Export ASCII Exports the active calibration date editor as ASCII.

Export HTML Creates an HTML file, the graph is saved using the same file name but the extension *.gif. The HTML files can be viewed using the internet explorer, thus, they can be used to save calibration results and to allow other users to download the files from a network system.

Print Prints the actual graph and the data editor.

Configurations Allows to load, modify and save the settings. The settings file contains the informations on the view and number of displayed columns, color scheme for data points as well as the color scheme of the axis. Upon loading a setting all parameters will be updated.

The Options menu allows for scaling operations and color setting of the active curve.

Autoscale Rescales the window such that all calibration points are visible.

Unzoom Unzooms a zoomed area back by one zoom operation.

Current Configuration A window opens, which allows to change the color and type setting for the active calibration. You also can define the selected settings to become settings for a different curve.

The Calibration menu allows the selection of different calibration procedures. It furthermore contains the Calibration settings. The selection of the appropriate fitting function however, is done using the Fit list.

Settings Allows to enter name of operator and internal standard, elution volume of internal standard, column name, Mark-Houwink parameters for calibration standard.

Polynomial Results Displays the polynomial coefficients, the minimum and maximum elution volume of the standards the least square fit χ^2 and the regression coefficient R.

Guided broad calibration Performs a broad calibration. The user is guided through the individual steps of this calibration procedure.

Guided integral calibration Performs a integral calibration. The user is guided through the individual steps of this calibration procedure.

Universal calibration Creates a new calibration from the active calibration its Mark-Houwink parameters and the Mark-Houwink parameters of the new substance.

The Table menu allows defining which columns will be displayed, and their view.

Column setup Allows to name the active column, and to adjust colors and text styles for column headings and column values.

Clear column Deletes the entries of the selected column.

Reset rows Eliminates empty rows after clearing rows.

Insert row Inserts a new row at the cursor position.

Delete row Deletes the selected row.

Clear row Deletes the entries of the selected row.

Data Editor Section

The data editor shows a variety of columns. The column design can be adjusted by activating the right mouse button on a column header. The columns can be sorted by any column information you like. Simply click on the column header using the left mouse button, and the all columns will be sorted according to the selected column in increasing (∇) or decreasing (Δ) order. The columns for residuals and slope of calibration curve will be automatically calculated for the active calibration when a fitting function was selected.

Upon activating the right mouse button within a cell of the data editor, a box is opened showing the same options as available from the Table menu.

Graphical Window Section

The Graphical window shows the calibration points of the active calibration, the fitted curve through the calibration points and the slope or residuals. The selection which calibration is displayed is done by the File list. The data editor section will always show the information belonging to the active calibration as shown in the File list.

If the overlay icon is activated all loaded calibration curves will be displayed. If a selection is made in the Compare list, the information for residuals or slope of the calibration curve of the active calibration will be displayed as well.

For the x- and y-axis the axis style can be selected, upon clicking the right mouse button on the respective axis. You can select the labels, the font and color of labels and numbers as well as the color of the axis region itself. Manual scaling of the axis is possible. By moving the mouse pointer into the x- or y-axis scaling region (upper left and lower right corner), arrows appear which allow to shrink and expand the x- or y-axis, respectively. The axes can be dragged as well. When moving the mouse pointer onto the axis, it changes its view to a pointing hand. Press the left mouse button and keep it pressed, while moving the axis. Then release it at the desired position.

You can zoom by simply selecting the first corner of the box using the left mouse button. Keep it pressed and move the mouse pointer to the desired position. The box will follow. Upon releasing the mouse button, the area within the box will be zoomed. If you want to switch back select Options > Autoscale.

When using the right mouse button within the Graphical window, a menu containing the items of the Options menu appears.

Creating a Narrow Standard Calibration Curve

In order to create a conventional narrow standard calibration curve, the elution volumes, molecular weights and statistical weights have to be entered in the Calibration window. Passing the respective information to the Calibration window directly from the Elugram can do this. Alternatively you can enter the data manually or import as ASCII data file. Immediately after input of the calibration point it is displayed in the graphical section of the Calibration window.

The data transfer can be started from the Agilent ChemStation standard data analysis or data files can be loaded directly in the Agilent GPC data analysis software. The later procedure is preferred if the manual processing of data files in the interactive screen review is preferred. The approach from the Agilent ChemStation is used when automated processing of samples and recalibration will be used (Chapter 3, “Introduction to the GPC Data Analysis Software”). This section describes the procedure working only in the interactive screen review.

1. Activate the Raw data window and load the first raw data file Raw data > load for the calibration and the default.cal Calibration data > load.
2. If you are working without internal standard, open the Calibration window, select File > New and proceed with step 9.
3. If you want to use internal standard correction of the elution volume, activate the Raw data window and move the cursor to the peak of the internal standard and look at the status area to read the elution volume as x value. It is not necessary to read the exact value at peak maximum.

If you have already performed an internal standard correction against a value given in a loaded raw data file (indicated by a light green marker for the internal standard) select Calibration Data > Information and note the value given under Internal Standard Calibration.

4 Open the Calibration window and load the calibration file DEFAULT.CAL.

5 Select Calibration > Parameters and enter the value you noted for the internal standard (step 2). Save the calibration curve under a new name (for example, Calib01.CAL).

6 Close the calibration file.

7 Load the calibration curve created under step 5 by activating the Raw data window and select Calibration data > Load.

8 Review the baseline and define the exact position of the internal standard using the functions of the x-axis (Functions of x-Axis, Internal Standard Search). Confirm the correct position with OK.

9 Change to the Elugram. Search for the peak maximum of the component peak by selecting Find Maximum from the functions of the x-axis.

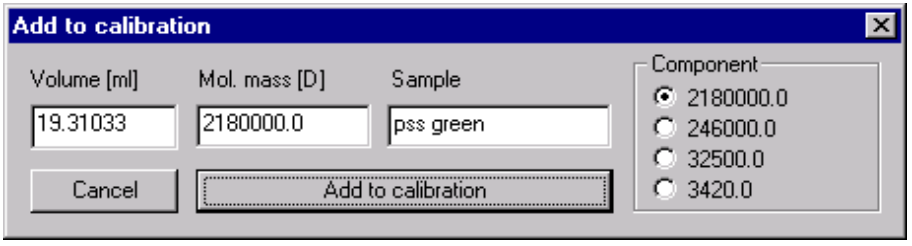


Figure 14 Add to calibration

10 In the Add to Calibration dialog box the peak maximum is shown. The molecular mass will be assigned to the peak maximum. You can either enter the molecular mass manually or select it from the component list. The component list includes the molecular mass you might have entered using the Agilent GPC data analysis software sample editor. Upon selecting Add to calibration the information concerning elution volume, molecular mass

and sample name will be transferred to the active calibration of the Calibration window.

11 Repeat step 1 and step 7 to step 9 for each calibration peak/calibration file.

12 Enter once again into the Calibration window. The calibration table should show the information on the calibration data points, while the graphical section should show the corresponding curve.

13 Fit the calibration points in the Graphical window by a suitable calibration function using the Fit list and check the parameters Calibration > Parameters (here the value noted in step 3 has to be entered again.).

14 Save the calibration curve.

15 To evaluate samples with the just created calibration curve, load it in the Agilent GPC data analysis software Program.

Unfortunately no analytical function exists which describes the shape of a calibration curve for all cases. The user must depend on intuition when adjusting the calibration curve to the calibration data. The quality of the adjustment can be determined by 3 criteria:

- The deviation between calibration points and calibration curve should be low, and randomly distributed. You can view the deviation within the respective column of the editor section and as graphical information by selection from the Compare list. To rely only on this requirement can easily create errors. For example, for 6 calibration points it is always possible to fit a polynomial 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 slope of the calibration 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.

Creating a Universal Calibration Curve

Activate the Calibration window and load your calibration curve Calibration > load, which you would like to be transferred into a new a universal calibration.

Select Universal Calibration, then Calculate Transformation and enter the Mark-Houwink coefficients for the new substance. If the Mark-Houwink coefficients of the original calibration curve are not correct, you have to correct them in the original calibration curve. The new calibration curve will be calculated when you select then OK and afterwards Yes.

Proceed now as if the calibration pairs would be created in the conventional way.

Creating a Broad Standard Calibration Curve

The guided broad standard calibration uses the active calibration curve as base calibration and transforms it into a new one such, that the average molecular weights calculated by the new calibration curve together with the selected chromatograms do match the expected values. For a more detailed description of background of broad standard calibration, refer to Chapter 2, “Basic Theory of GPC” or to the literature. The software will guide you to create a broad standard calibration curve.

- Make sure the narrow standard calibration curve is available that you like to use and the data files for the broad standards are saved as overlays (*.add).
- Activate the Calibration window and load the narrow standard calibration curve. Select Calibration > Guided broad calibration.
- Press OK to confirm that the active calibration curve will be used as base calibration. If this calibration curve is not correct, select Cancel to leave the guided broad calibration and select the correct calibration as active calibration.
- In step 1 you will be forced to load a valid overlay file or to use the actual elugram data. This overlay file can contain up to 8 elugrams. The average molecular weights of these chromatograms can be fitted simultaneously. The creation of overlay files is described in the “Menu Overlay” on page 77.

- In step 2 you choose which kinds of averages you will use (M_n, M_w, [η]). Enter the corresponding averages and select a statistical weight of 1 if you don not have any reason to give a higher or lower statistical weight to any of the entered averages.

NOTE If you are using the intrinsic viscosities ([η]) the Mark-Houwink coefficients of the original base calibration have to be correct. You can correct these within the step 4 of the broad calibration routine.

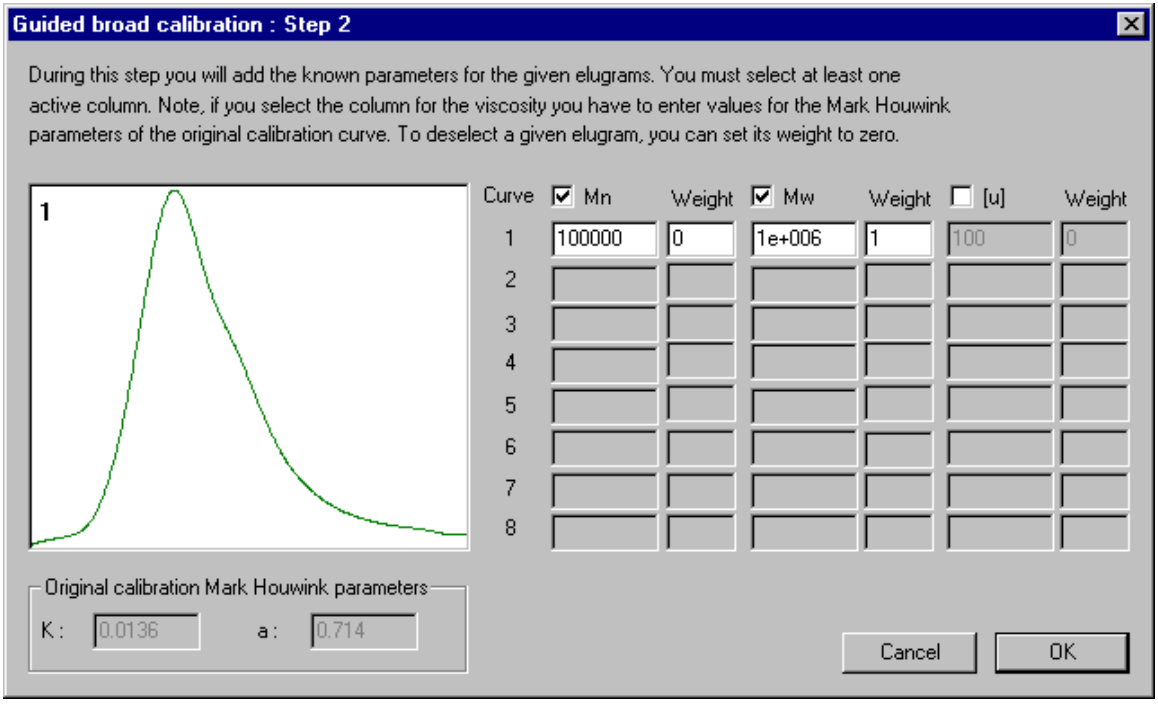


Figure 15 Guided broad calibration: Step 2

In step 3 the fitting procedure will be performed. The calibration program first performs a raw search for a useful starting point of the fitting routine. If you select skip in the grid evaluation section of the window, the start parameters used are identical to the start parameters entered in the start section.

Otherwise the program performs a grid evaluation within the limits given in the fit parameters section, in order to find a good starting point for the actual fitting procedure.

Using the Start button, the fit will be performed.

The Calibration window displays the target values and actual values for the molecular weights in the upper part of the window, while in the lower part you will find the deviation (sum of the squared relative deviations of target and actual value). The fitting procedure will take some time dependent on the complexity of the 3-dimensional surface. You can stop the evaluation anytime using the stop button. If the fitting limits are reached by one of the parameters the fitting process will be interrupted. You can then change the fitting limits, and can start the evaluation again, or go on with the evaluation starting at the present set of parameters after entering them in the start boxes and using the skip option within the grid evaluation section.

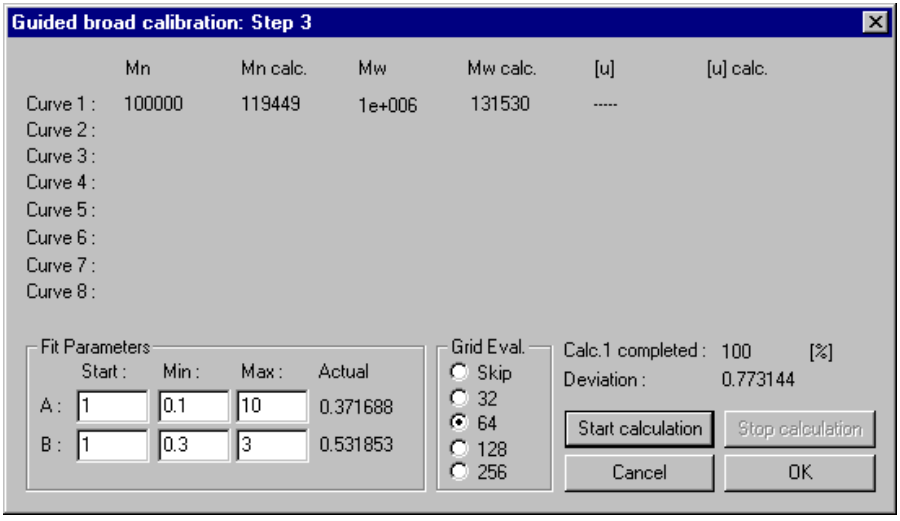


Figure 16 Guided broad calibration: Step 3

After the fitting procedure is completed use the OK button to continue to the next step.

The parameters A and B fitted in the fitting routine can be expressed by the Mark-Houwink coefficients of the base calibration and the substance for which the calibration curve has to be created (s. theoretical aspects of broad

calibration). The actual window allows to calculate the Mark-Houwink coefficients of the substance under investigation using the Mark-Houwink coefficients of the base calibration and the fitting parameters A and B. The Mark-Houwink coefficients entered for the base calibration are shown in the Given section. If these are not correct, enter the correct ones. You can now recalculate the new Mark-Houwink coefficients. If you go on using the OK button, you have to decide, if you want create a new calibration table. If you choose Yes, the Calibration window will transform the base calibration into a new one. The molecular weights of the base calibration are replaced by the molecular weights calculated from the base calibration and the fitting parameters A and B. Furthermore the calculated Mark-Houwink coefficients for the substance under investigation will be copied into the calibration of the new calibration curve.

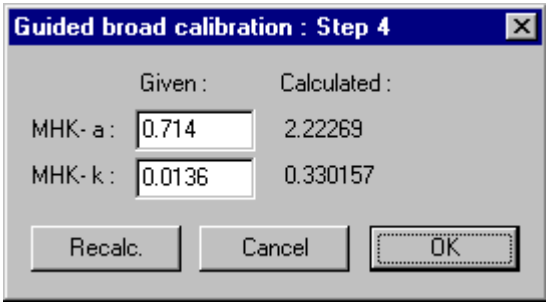


Figure 17 Guided broad calibration: Step 4

The calibration points of the new calibration can now be fitted as described in Chapter 3, “Introduction to the GPC Data Analysis Software”.

Creating an Integral Calibration Curve

There is a fundamental difference between the integral calibration procedure and others In contrast to the other calibration procedures which transforms one calibration curve into a different one, thus automatically creating a new calibration curve, the integral calibration procedure does not automatically create a new calibration. If integral calibration is selected while an existing calibration is active, the data points created by integral calibration procedure will be added to the active calibration. Thus, if a new calibration should be created, you will have to start from an empty calibration curve (File > New).

The integral calibration will be guided in order to make it as convenient as possible.

The integral calibration requires an overlay (*.ADD) obtained on the column that has to be calibrated. Furthermore the integral (cumulative) molecular weight distribution of the sample has to be known, at least for a few data points.

Use File > New to open an empty calibration, otherwise the data points will be added to the active calibration. To perform the guided integral calibration procedure select Calibration > Guided Integral Calibration.

You will be asked to load the overlay file. This file may contain up to 8 elugrams. One of those has to be the one that has to be used for the calibration procedure.

In step 2 you will have to select the curve that you want to use for the calibration procedure. Only for the selected curve the integral calibration will be displayed.

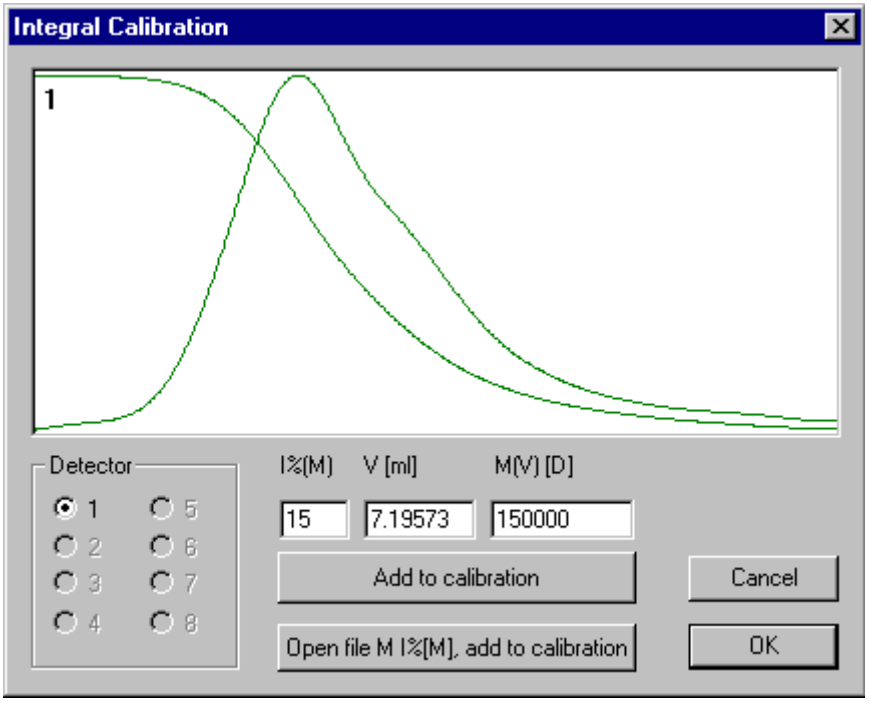


Figure 18 Integral Calibration

Enter the different pairs of molecular weight and integral distribution. When the value for the integral molecular weight distribution is entered, the corresponding elution volume is displayed immediately. Use the Add to Calibration button, to add the desired data point to the calibration. As an alternative, you can load an ASCII file (creation see below), which contains the columns molecular weight and integral molecular weight distribution, using the button Open file M, I% (M), add to calibration. The calibration program will now find the elution volumes for all pairs of molecular weight and integral calibration. Within one session, you can change the curves within an ADD file, can load new ADD files and the corresponding ASCII files. When you have processed all your data, leave the window for integral calibration using the OK button.

The data points can now be fitted as described in Chapter 3, “Introduction to the GPC Data Analysis Software”.

Typing the molecular weights into the first, the value for the integral distribution into the second column of the calibration editor, and exporting these data as ASCII file can easily create ASCII files for integral calibration.

6
Appendix

Relation between Curve Color and Line Style in Monochrome Printing

Table 4 Numbers in order of the selection box, from left upper side to right lower side

1	Old white	Middle-short-middle
2	Black	Short-short-short
3	Gray	Middle-middle-middle
4	Darkgray	Middle-short-middle
5	Red	Straight
6	Dark red	Middle-middle-middle(no distance)
7	Yellow	Long-long-long
8	Ochre	Dotted
9	Green	Middle -dot- middle
10	Dark green	Long-long-long
11	Turquoise	Long-short-long
12	Dark turquoise	Middle-middle-middle
13	Blue	Long-short-long
14	Dark blue	Long-short-long
15	Purple	Short-short-short
16	Dark purple	Straight
17	Light green	Dotted
18	Light blue	Middle-middle-middle (no distance)
19	White	Middle-dot-middle
20	Gray	Straight

System Verification

Experimental Conditions

Polymer Type	Polystyrene
Eluent	THF
Temperature	25°C
Calibration Std	Polystyrene

Mark-Houwink Coefficients

K [ml/g]	0.01363
A	0.714
Concentration	4.343 g/l

Theoretical Results

M_n = 150000 D

M_w = 300000 D

M_z = 450000 D

D = 2.0

M_v = 280869 D

eta = 105.67 ml/g

Default Settings

Default Calibration File (def.cal)

Calibration bound min	0.5 ml
Calibration bound max	300 ml
Internal standard position	50 ml

Mark-Houwink Coefficients

K [ml/g]	0.0
A	1.0

Default Acquisition Parameters

Flowrate	1 ml/min
Inject Volume	20 µl
Temperature	23°C assumed in case no sample data are available

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7 References

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In This Book

This manual will help you to familiarize yourself with the Agilent Technologies GPC data analysis software. Further, the manual describes in detail all the features and evaluation parameters of the software.

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