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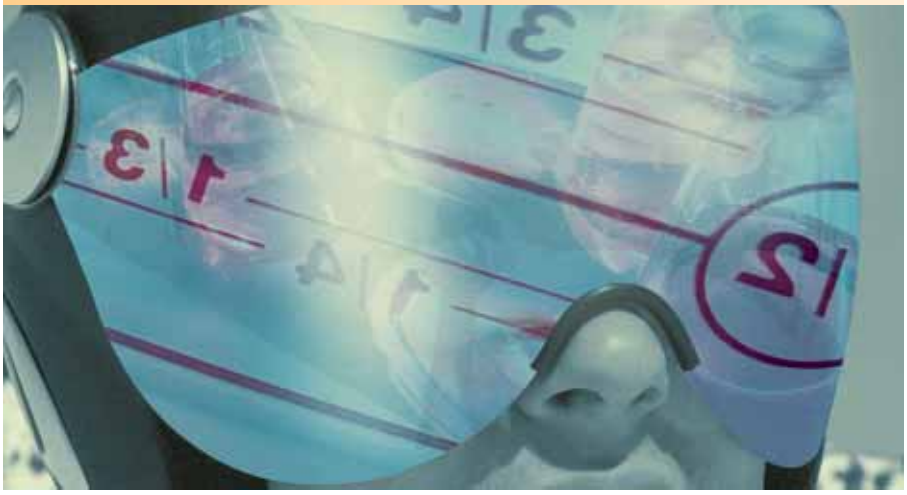
GPC Streamliner

Focus: accurate GPC/SEC



Even with the most modern GPC/SEC equipment, method know-how is essential for producing accurate and precise results. PSS has focused on GPC/SEC for more than 20 years, specifically on the fundamental knowledge of GPC/SEC basics, the column chemistry, and the development of all-around solutions to answer GPC/SEC questions. Our day-to-day experience and cooperation with scientists all over the world has provided the know-how that facilitates the development of new column applications and software solutions. The main features in this issue of the GPC Streamliner are a method application for the characterization of natural starches, and a software tool for sieve curve determination in membrane characterization. In addition, to support GPC/SEC users, we cover the PSS GPC/SEC training courses and the PSS "GPC/SEC Tips and Tricks", a column developed by PSS and published in "LCGC" e-magazine.

In This Issue

Sieve Curves

Membrane characterization using GPC/SEC

One filtration experiment followed by GPC/SEC measurements is sufficient to produce a comprehensive sieve curves analysis, using standard equipment already available in many analytical labs.

GPC/SEC allows the reliable and fast measurement of important membrane properties such as:

- Average pore size
- Pore size distribution
- Molar mass cut-off
- Membrane selectivity
- Retention efficiency
- Pore accessibility
- Membrane stability
- Hydrophobic properties

The sieve curves can help identify low quality membranes and establish quality parameters for working membranes.

Sieve Curve Determination Technique:

The membrane to be characterized is used to filter a stock solution of a broad molar mass/molecular size distribution reference standard. The stock solution, the filtrate and optionally the retained fraction (retentate), are analyzed on a typical GPC/SEC system, for example, the PSS SECcurity GPC1200 system, which uses the WinGPC Unity Macromolecular Chromatography Data System (MCDS).

A direct comparison of the two (three) GPC/SEC chromatograms shows the retention for every molar mass and pore size diameter. Narrow molecular weight standards of the same type, e.g. a set of PSS Pullulan standards with different molecular weights, provide a calibration curve, which is required for molar mass evaluations. The calibration can be obtained using WinGPC Unity MCDS, which supports all calibration methods.

An easy-to-use curve dialog in WinGPC permits the simultaneous determination of various sieve curves from different filtrate/retentate samples. Many labora-

» Continues on Page 2

Membrane characterization

» From Page 1

Table 1:

	UF Membrane		
	1	2	3
Mw cut-off [Da] (50% retention)	Not defined	12 400	11 800
Mw cut-off [Da] (80%)	41 200	48 800	49 500
Mw cut-off [Da] (90%)	79 600	87 500	105 000
Mw cut-off [Da] (95%)	124 000	184 000	284 000
Mw cut-off [Da] (99%)	352 000	352 000	502 000
Average pore size [nm]	8.8	10.8	11.0
Membrane selectivity	Not defined	7.6	7.8

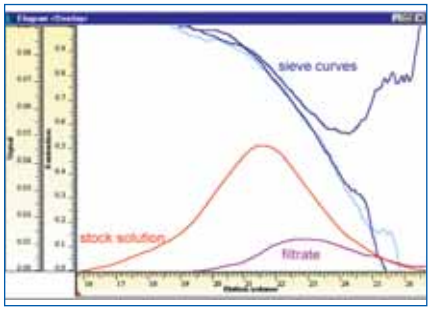


Fig. 1: Overlay of stock solution and filtrate (only one) GPC/SEC chromatograms. The resulting sieve curves for all three UF membranes for water treatment are displayed too

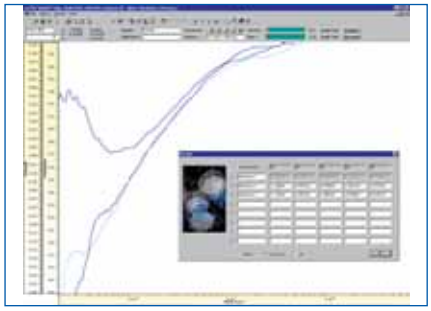


Fig. 2: Comparison of the three UF membrane sieve curves and their retention behavior with molar mass. The molar mass cut-off values (user-selectable) are also shown

tories have their own calculation procedures for sieve curve determination from stock (s), filtrate (f), and retentate (r, optional). WinGPC allows the use of a sieve curve determination formula to be applied, when a different method is used. The same is true for the calculation of molecular size from molar mass. WinGPC accepts custom relations that describe the correlation between molar mass and pore size.

The resulting sieve curves are displayed in the elugram window with elution volume axis as well as in the molar mass distribution window with log M axis. The numerical results for up to 5 molar mass cut-off values are summarized in the results window, expressed either as elution volume, molar mass, or pore diameter.

The WinGPC Report Designer offers a dedicated sieve curve report with a summary of all important results. This report can be modified with additional results, company logo or other information. Alternatively results can be exported to other applications (e.g. Word or Power Point).

The following example shows sieve curves for three different ultra filtration (UF) membranes used for water treatment. The final goal was to establish a fast and reliable method to identify low quality membranes during the quality control.

Experimental conditions:

System:	PSS SECcurity GPC1200 with RI detection
Stock solution:	Broadly distributed PSS Pullulan Membrane Standard (linear sample)
Eluent:	Water, NaN_3 0.05%
Columns:	PSS SUPREMA 10µm 30 Å + 1000 Å + 1000 Å (8 x 300 mm each), SUPREMA precolumn
Calibration:	PSS Pullulan calibration kit
Data System:	PSS WinGPC Unity MCDS SR1, Build 5403

Fig. 2 shows an overlay of the three UF sieve curves. Membranes 2 and 3 show the expected behavior and similar characteristics. Membrane 3 is easily identified as out-of-spec product. Table 1 summarizes the numerical results for 5 different molar mass cut-off values, average pore size, and membrane selectivity as calculated by WinGPC Unity.

Conclusion:

GPC/SEC is useful for the characterization of membranes and determination of membrane separation parameters. Comprehensive sieve curves can be determined with one filtration experiment and two measurements using standard equipment already available in many analytical labs. The sieve curves can help to identify low quality membranes and to establish quality parameters for working membranes.

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Announcement

PSS Catalog of Reference Polymer Standards & Separation Columns: much more than a product list

PSS' 2007 catalog shows a large selection of molecular weight reference polymer standards, calibration kits and specialty polymers for organic and aqueous solvents. It offers an unparalleled selection of compounds and molecular weights. In addition it includes carefully selected calibration kits with precisely characterized individual standards as well as the original ReadyCal calibration kits for fast calibration of GPC/SEC systems. Validation kits for the validation of instruments and detectors complement the comprehensive product line. The catalog also features a range of column materials, perfectly suited for all GPC/SEC solvents and samples, resulting from product development at PSS. Detailed application examples facilitate the selection of the correct material while valuable practical information help select the optimum particle size and porosity for highest resolution.

New staff in Mainz

PSS invests in new employees. Since 01.01.2007 Thomas Fickinger joined our sales and marketing team in Germany. He is responsible for GPC equipment and molar mass sensitive detectors as well as software, GPC columns and reference materials. He is also a competent contact person for our analytical services customers. Thomas Fickinger has ten years of experience in the field analytical equipment.

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NetCommunity on www.polymer.de

The „PSS NetCommunity“ offers its members a series of free services:

- **application database:** This database provides comprehensive method information as well as chromatograms and discussion of results for many GPC/SEC applications. Customers can search for polymer type and group as well as for column material and solvents.
- **literature database:** PSS NetCommunity members can search for literature featuring PSS products or for technical articles with comprehensive information on on all GPC/SEC related methods. Many pdf documents are ready for download.
- **WinGPC software database:** Software tips and tricks, applications and feature articles for WinGPC users can be found in this database.

Please register online at www.polymer.de

PSS works on a relaunch of www.polymer.de. The NetCommunity will be an essential part of new homepage. NetCommunity members will automatically be pre-registered for the new website and informed about the release date.

Characterization of natural starches with GPC/SEC

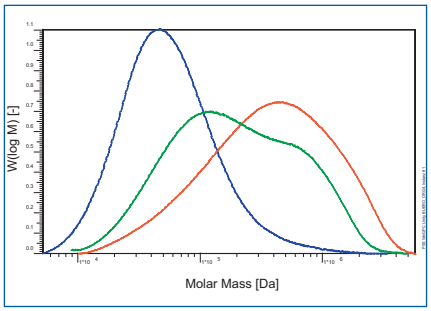

Fig. 1: Separation of Amylopectin, Amylose and potato starch on PSS GRAM columns (30 000 Å 10 µm 8 x 300 mm + precolumn). Conditions: eluent: DMSO, LiBr 5 g/l; temperature: 60° C, flow rate: 0.5 ml/min; concentration: 0.5 g/l

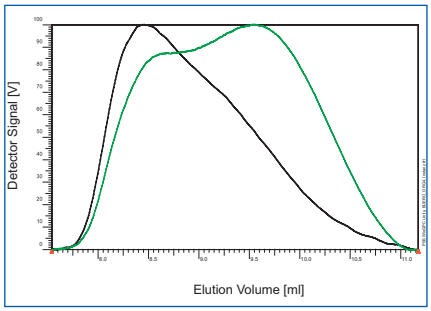

Fig. 2: Analysis of potato starch with RI detection (green curve) and 90° light scattering detection (black curve)

Starches are energy storage or reserve polysaccharides. Other examples of natural polysaccharides that function as energy release and reserve substances are dextrin, fructan and glycogen (in animals). Other functions of polysaccharides in nature are as structural agents like cellulose, pectin (in plants); chitin, etc. and water storing agents like carrageenan, agar, pectin in plants, and glycogen in animals

Starches are used in large quantities for the swelling and adhesive industry, the pharmaceutical industry and the biotechnology.

Starch consists of two macromolecules which differ in size and structure:

- 1. Amylose (15-30 w%) is partly branched with molar masses (Mn) between 50 000 and 150 000 g/mol
- 2. Amylopectin (70-85 w%) is a highly branched macromolecule with molar masses up to approx. 3 x 10^7 g/mol (this is equivalent to 2 x 10^5 glucose units).

The characterization of natural starches is always a challenge for the analyst since starches have:

- numerous reactive resp. functional groups (-OH; $-CH_2OH$ and -O-) in the chains
- very high molar masses
- very complex structures

GPC/SEC in combination with molar mass sensitive detection is commonly used to (first) separate starches by size and to (second) characterize the substances comprehensively.

A typical molar mass sensitive detector is a multi angle laser light scattering detector (MALLS). This detector has the advantage of providing structural information in addition to the molar masses. However, to make full use of this advanced characterization method it is necessary to develop an interaction free GPC/SEC method, which relies on an optimum stationary phase (column material) and matching eluent system.

Sample preparation:

Sample preparation is a crucial step for successful GPC/SEC separation / analysis of starches. Starches are colloidal soluble in hot water, they are susceptible to sample degradation during dissolution. To prevent starch degradation, DMSO constitutes a good alternative solvent for GPC/SEC. While starches are completely soluble important properties are kept. The samples are dissolved at 80° C in DMSO for 8 hours, after that the solutions are heated to 120° C for 4 hours. Starch denatures under these conditions, i.e. it loses partly the sheet structure and the helical structure.

Results:

Fig. 1 shows the separation of three different starches on PSS GRAM columns in DMSO. The calibration with Pullulan standards allows the relative determination of the molar masses and the determination of the molar mass distribution. The quality of the sample preparation procedure can be checked using a 90° light scattering detector, e.g. the PSS SLD1000 and an RI detector. Undissolved higher molecular weight parts should be easily detected by the molar mass sensitive LS detector, even when the concentrations are very low. Sample preparation has been successful, when the SLD1000 does not detect peaks at very low elution volume. If the RI detector, as shown in this case, also does not detect any low molecular weight peaks at high elution volume sample degradation can be excluded.

On the other hand the combination of light scattering and RI detection also helps to check if the GPC/SEC separation is really interaction free and according to molecular size (see Fig. 2). It is inevitable for any GPC/SEC mechanism that the molar mass is decreasing with the elution volume. If the on-line measured molar mass does not show that behavior it can be concluded that the column material is not the best choice for the application. In case of PSS GRAM material it could be shown that starches show the expected GPC/SEC elution behavior.

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Innovations

Polyolefin characterization

More than 20 years of experience in high temperature GPC enable the PSS contract analysis laboratory to continuously expand the application range. PSS offers GPC polyolefin measurements at 140° C (e.g. for Polyethylenes) and at 160° C (e.g. for Polypropylenes). If required the measurements can be performed according to ASTM Designation D6474-99.

On-line GPC coupling with viscosity detection is recommended for structural or branching information, whereas GPC coupling with off-line FTIR-detection is levant for product deformulation and substance identification.

The analytical report will contain the raw data and results (elugram, molecular weight distribution), as well as measuring conditions. It is usually e-mailed in electronic format (pdf); ASCII data and slice results are available on request.

WinGPC Unity Service Release 1

The WinGPC Unity Service Release SR 1 features new functions and offers full support of the new modules: SystemPilot for convenient control of Agilent 1100/1200 systems via WinGPC; Compliance Pack for FDA 21CFR11 compliance. Furthermore the automatic test routines and installation qualification (IQ) routines of the PSS Universal Data Center UDC810 are fully integrated. A change control document comes with every delivery and is available on request before ordering.

WinGPC Unity SR 1 is free for all WinGPC Unity users with build number 2488 and above.

All other users can update to WinGPC Unity SR 1.

Part number: 400-0201
for updates from WinGPC versions < 6
Part number: 400-0202
for updates from WinGPC version 6

New Degassers for GPC/SEC

Special online vacuum degasser for GPC/SEC

Specifications:

- 2 independent eluent channels, may be upgraded to 4 eluent channels
- small inner volume (480 µl)
- optimized degassing up to a maximum flow rate of 3 ml/min per channel
- suitable for all GPC/SEC solvents including HFIP

Part number: 409-0024

GPC/SEC courses and advanced user training

For more than 20 years PSS offers training courses for beginners and advanced users of GPC/SEC. Starting with German courses in the late 80s, the program has been expanded to English courses that can be booked in Mainz, Germany, or in-house at customer site.

The general PSS strategy is to reinforce the knowledge gained during the morning theoretical sessions with hands on practical sessions in the afternoon. This approach has been highly rated by more than 700 participants trained over the last years.

The following training courses are available:

GPC/SEC training course

This course, that takes place in Mainz, Germany, provides theoretical lectures and practical sessions for modern analysis of macromolecules using gel permeation chromatography (GPC), also known as size exclusion chromatography (SEC). It covers the separation technique, gives practical advice for reproducible and accurate analysis, and shows the application advantages as well as the limitations of GPC/SEC and GPC/SEC with light scattering detection, viscometry detection and other techniques. The number of participants is limited; practical sessions are restricted to a max. of 5 people per instructor. You may choose to join groups working with aqueous or organic solvents and/or between groups for beginners and advanced users.

The first day usually covers the principles of GPC/SEC:

- GPC/SEC Separation mechanism
- Molar mass distribution and averages: determination and meaning
- GPC/SEC instruments and detectors
- Method development, column selection
- Trouble shooting

During the practical session a GPC/SEC equipment is installed and tested. Important column parameters as resolution, plate count, and asymmetry are measured.

The second day introduces different GPC/SEC calibration methods and their applications:

- What calibration technique is best for my application?
- When should universal/broad/integral calibration be used?
- How do I calibrate my system to analyze copolymers/branched polymers?
- What is the influence of different experimental parameters (sample concentration, flow rate, and temperature) on the GPC/SEC separation?

The practical session reviews calibration curves and different calibration methods. Furthermore the influence of evaluation parameters is measured and discussed.

Upcoming courses:
11. – 12.10.2007 in Mainz, Germany
28. – 29.02.2008 in Mainz, Germany
09. – 10.10.2008 in Mainz, Germany

Detailed programs and registration forms are available under www.polymer.de

Part number: is 899-0022

Customer based training

PSS offers the training course also as in-house training on customer site, for analytical labs with many employees. Additionally, customers are welcome to discuss the program and their special application needs with a chromatography expert, before the training, to provide an individual tailor-made training focus.
While not a must, the training can include a WinGPC software training session as well. The practical part can also be adapted to the methods applied in the lab: e.g. conventional GPC/SEC, GPC/SEC-Viscometry, GPC/SEC-Light scattering.
Appointment on request

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Upcoming events

GPC training courses in Mainz, Germany:

See beside

Shows and Exhibits

02.07. – 06.07.2007
European Polymer Congress; Portoroz, Slovenia
Talk Peter Kilz: Elucidation of Macromolecular Architecture by Chromatography with Mass- and Structure Sensitive Detectors
Please visit our booth

05.08. – 11.08.2007
41st IUPAC World Chemistry Congress; Torino, Italy
Talk Peter Kilz: Investigation of the molecular properties of starches for food applications by SEC-MALLS

19.08. – 23.08.2007
234th ACS Meeting & Exposition; Boston, USA
Booth: 1042
Talk Dr. Thorsten Hofe: Non-invasive studies of proteins and DNA fragments by SEC and SEC coupling methods (20.08.07 2.30 –2.50 pm; Division of Polymeric Materials: Science & Engineering, Polypeptide and Protein Materials session)

02.09. – 07.09.2007
International Symposium on Ionic Polymerization 2007; Banz Monastry (Bayreuth), Germany
http://www.bayceer.uni-bayreuth.de/ip07/
Please visit our booth

09.09. – 11.09.2007
Bayreuth Polymer Symposium; Bayreuth, Germany
Please visit our booth

25.09. – 28.09.2007
Ilmac; Basel, Switzerland
Booth: A91 in exhibition hall 1.1

04.10.2007
DSP-Meeting; Amstelveen, Netherlands
Talk Dr. Günter Reinhold: Column Selection for true GPC and possible error sources

01.04. – 04.04.2008
Analytica 2008; München; Germany

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GPC/SEC Tips & Tricks

How to understand molar mass distributions?

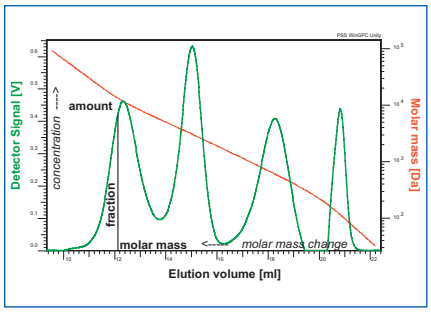

Fig. 1: GPC/SEC chromatogram of a sample mixture (green) with overlayed calibration curve (red)

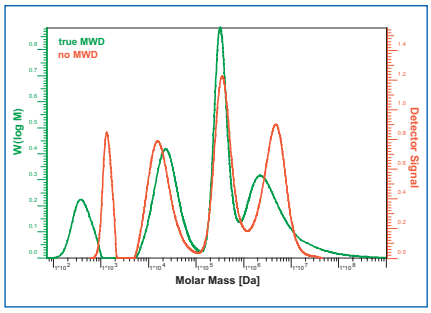

Fig. 2: Overlay of a real (green) with a molar mass distribution (red), wrong calculated by a HPLC data system

Question:

What is special about molar mass distributions and what is the difference between a chromatogram and a molar mass distribution?

Answer:

Unlike low molecular weight substances, synthetic materials, polysaccharides, proteins, etc. do not exhibit a definite molar mass. They consist of mixtures of chains with different numbers of repeating units, so that all chains possess their own molar mass. The molar mass(es) of a macromolecule is obtained by averaging the molar mass of the different chains by number (Mn) or by weight (Mw). However, even with Mn, Mw and the polydispersity index D (Mw/Mn) macromolecules are not characterized comprehensively. They can have the same averages but still show significant different physical properties. This is then due to the fact that they have a different molar mass distribution meaning that the fractions of the defined molar masses are different.

Molecular weight distributions can be measured with separation techniques (mostly GPC/SEC). GPC/SEC chromatograms (compare Fig. 1) show the fractions and the change of the property values for the sample, but this information is overlayed with parameters of the analytical equipment.

A thought experiment makes clearer what that means: the same sample is measured in two laboratories on two different equipments with different sized columns. The resulting chromatograms will look different and without previous knowledge nobody will assume, that the chromatograms represent the same sample.

However, when the samples are correctly evaluated, e.g. using any kind of calibration (conventional, universal, light scattering), the influence of the equipment is eliminated. The resulting molar mass distributions should be the same, since they belong to the same sample. Thus molar mass distributions allow unaltered information and offer a direct comparison of product specifications.

How are GPC/SEC chromatograms transformed into molar mass distributions?

1. The chromatogram shows a concentration distribution curve, where the molar mass decreases with higher retention times (elution volumes).
2. First the retention axis (x-axis) will be changed into a molar mass axis via a molar mass calibration.
3. In the second step the y-axis is converted in mass fractions (in one molar mass interval) w(lg M). This is necessary, because signals in a chromatogram are recorded in a constant time interval. However for the molar mass distribution the concentration in a constant molar mass interval is necessary.

Unfortunately many HPLC data systems with addons for GPC/SEC calculate „molar mass diagrams“, where only the retention times are converted into molar masses. This can make the inter laboratory comparison extremely difficult. Also the determination of fractions above/below certain molar masses, e.g. below 500 g/mol, can be faulty. Fig. 2 shows clearly, that in addition peak position (molar mass) and peak width (polydispersity index) can be wrong. Are the averages of the molar masses also wrong? No, most of the time this is not the case. Usually the averages are not calculated from distribution curves and are therefore unaffected by this phenomenon.

An easy test shows if molar mass distributions or “molar mass diagrams” are shown:

Inject a mixture of polymer standards with the same concentration on a GPC/SEC column (no linear or mixed bed column) and generate a non-linear molar mass calibration using any polynomial function (e.g. cubic fit, polynomial 3, see Fig. 1). Analyze the standards mixture to obtain the molar mass distribution. If peak heights and peak widths do not vary, your data system does not show molar mass distributions but only molar mass scaled chromatograms (Fig. 2).

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Application

Characterization of Polyether ether ketone (PEEK)

Polyether ketones represent a special class of high performance polymers with extremely high melting points, e.g. PEEK: 334° C. First synthesized in the end of the 70's they convince with extraordinary chemical resistance. As partly crystalline thermoplastic resins they exhibit a broad application range in aerospace industry as well as in laboratories. However for the analytical chemist the high chemical resistance is a problem, since the choice of a suitable solvent is extremely difficult.

Sample preparation:

The sample is dissolved in a 1:1 mixture of phenol and 1,2,4-trichlorobenzene for 12 hours at 220° C in an autoclave. The high temperature of 220° C has to be employed to break the crystalline structures. After the PEEK is dissolved, it stays in solution above 150° C.

Analytical conditions:

Eluent:	1,2,4-trichlorobenzene/phenol 1:1
Columns:	PSS POLEFIN 10 µm 1000 Å + 100 000 Å + 1 000 000 Å (each 8 x 300 mm) + precolumn
Calibration kit:	PSS ReadyCal Poly(styrene) (M_p: 376 – 2 570 000 g/mol)
Data acquisition:	PSS WinGPC Unity
Detector:	RI Waters 150C
Fow rate:	1.0 ml/min
Concentration:	1.6 g/l
Injection volume:	150 µl
Temperature:	160° C

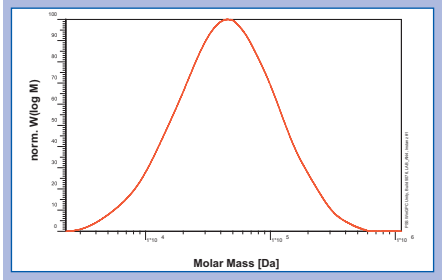

Fig. 1: molar mass distribution of a PEEK sample

Results:

The molar mass (M_n: 29 000 g/mol, M_w: 65 000 g/mol) and the polydispersity of 2.2 lie in the typical range of polycondensates (theoretical: PDI=2,0).

Conclusion:

In comparison to traditionally used solvents like dichloro acetic acid or sulfuric acid the mixture of phenol/trichlorobenzene represents a relatively gentle system. Chromatographic equipment is prevented from damage with these less corrosive solvents. Regular columns can be applied and the dn/dc-value is high enough, so that RI detection is possible. Therefore any high temperature GPC system can be used for measuring polyether ketons.