Mass matters!

Mass is an important property, not only in chemistry and physics. When hearing the word “mass”, PSS employees and customers’ first thoughts are related to the determination of the molecular weight of macromolecules. And, of course, the precise and accurate determination of macromolecular mass with a variety of methods, is still one of PSS’ most important goals.

However, in 2011 PSS will not only rely on well-known methods for molar mass determination, but will also break new ground. With a focus on mass spectrometry, which has been becoming more important recently for GPC/SEC, we will explore new solutions. Therefore this edition of the PSS Streamliner concentrates on mass spectrometry and provides you with an overview of all the PSS solutions. This includes not only the description of our new WinGPC software module for mass spectrometry, but also features articles about analytical services at PSS and new developments with PSS columns. In addition we present a field report of an experienced GPC/SEC-ESI-MS user with a lot of practical tips for setting up and running a system successfully.

Besides the molar mass of macromolecules, PSS employees at our headquarters in Mainz had also to deal with an issue of critical mass. Our current facilities, originally constructed in 2001, became too small for all the new employees and increased production capacities we have experienced. Therefore PSS started to build an extension in 2010. In January 2011 the PSS staff moved into these new offices. An interview with Joachim Kilz provides you with detailed information and shows you why this enables PSS to remain your reliable partner for all areas of GPC/SEC.

Since our new expansion also provides additional training rooms, we want to invite you personally to participate in one of our training courses scheduled this year. If attending a course in person in Germany poses a logistical problem, we have added a full schedule of new web-based trainings for anyone who might benefit from additional exposure to theory and practice in a wide variety of GPC/SEC techniques. More information can be found on page 8 or at www.polymer.de.

A brief review of our latest column seminar 2010, with the new developments for GPC/SEC columns can be also found in this Streamliner. PSS will offer this seminar in English in November 2011. Please do not forget to register soon, since space is limited.

Yours

Daniela Held
New Software Solutions
Quantitative analysis of macromolecules by GPC/SEC-mass spectrometry

The recent development of Mass Spectrometers (MS) with efficient and gentle ionization, has opened the door for new solutions and devices to characterize macromolecules via the hyphenation of GPC/SEC with MS. Research groups such as Karlsruhe Institute of Technology (KIT), National Institute of Standards and Technology (NIST), BAM, etc., have developed powerful analytical methods to successfully investigate polymers with MS; what was lacking until now, was a software solution designed for non-MS-experts. This is no longer the case; PSS has developed a Software Module which provides an elegant integration of GPC/SEC and MS analysis, empowering most analysts to obtain comprehensive information about new and complex macromolecules quickly and easily.

The next release of PSS’ Macromolecular Chromatography Data System, WinGPC 8, will reflect the latest developments in mass spectrometry, offering for the first time a MS module for the quantitative analysis of polymers by GPC/SEC-MS coupling. The WinGPC MS module is especially suitable for, electro-spray ionization mass spectrometry (ESI-MS) coupling - in our opinion the most versatile technology for macromolecules - and supports other techniques such as MSn and MALDI. This software development was achieved in cooperation with the group of Prof. Christopher Barner-Kowollik from the Karlsruhe Institute of Technology (KIT).

GPC/SEC-MS Coupling Setup

The online connection of a MS to a standard LC system is easily done using a T-connector, with the main flow going through the concentration detector (Figure 1). The MS itself is controlled by the MS software, which also records the MS data. After the measurement is finished, the MS data is imported into the WinGPC 8 database for review and evaluation at any time, without the need for further imports.

After the spectra are imported into WinGPC, the MS detector signals are treated in the same way as any other detector signals. First, the inter-detector delay for each detector has to be entered, so that the correct physical slice information (e.g. concentration of the selected group, MS spectrum, viscosity, molecular weight from an online viscometer or a conventional calibration curve, radius of gyration from MALLS, etc.) can be used for investigation. The detailed analysis starts after setting of the baseline and integration limits.

By setting a green marker in the new MS window to a dedicated elution volume, the associated mass spectrum of this slice is shown (Figure 2). Due to the high resolution of modern MS instruments, it is possible to learn more about not only the distribution of the main components, but also to see secondary distributions like: 
- Species with different end groups and cycles
- The existence of chemically defined or complex macromolecules
- Copolymers beside homopolymers
- Homopolymer contamination in copolymers
- Branch points of star and comb polymers
- Analysis of chain termination in polymer synthesis
- Information on chain degradation during aging/stress
- Out-of-spec tests

WinGPC MS window: The upper part shows the detector signals (in this case, the RI signal (blue) and the MS signal (TIC, total ion count, red); the lower part shows the mass spectrum at the elution volume of the green fraction marker with automatic assignment of charged state, degree of polymerization and molecular weight of the end group.
Although an advantage of ESI-MS is the possibility of the occurrence of multiple charges, it complicates the data interpretation. However, with WinGPC 8, once simple experimental parameters are entered (Figure 3) the typical MS-analysis of homopolymers and copolymers are done automatically. WinGPC determines the charged states of all ions, and shows for each MS peak in this spectrum, the charged state, the chain length/degree of polymerization and the mass of the end group.

**Fig. 3**

**MS parameters:**
- For homopolymers, the molecular weight of the repetition unit and the molecular weight of ionization agents is needed. In our example, a PMMA is investigated with NaI.
- For copolymers also the molecular weight of the second monomer has to be entered (in the example: none, because it is a PMMA homopolymer).

**Determination of absolute molecular weights**

By selecting a specific structure or the base peak (most abundant signal in the spectrum), it is possible to display the distribution of this structure as a chromatogram. This yields the Extracted Ion Chromatogram (EIC), sometimes also referred to as Single Oligomer Profile (SOP). Each of these structures generates its own matching calibration, because the MS allows the absolute determination of molar masses very precisely. For the determination of the molar mass distribution, not only the molar mass from the MS itself is important, but also the concentration of a species is required. This cannot be determined from the MS, because MS signals cannot be quantified easily. Therefore concentration detectors (RI, UV, ELSD) are used to determine the concentration of all species (structures), just like with the coupling of viscometry or light scattering detectors with concentration detectors.

The combination of MS and concentration detection allows determining the molar mass distribution and the true molar mass averages for each structure without calibration with reference materials or the additional knowledge of sample related parameters (e.g., dn/dc values for copolymers). Through selection and combination of different structures also the correct molecular weight of a subset of the total sample can be determined. An additional benefit from system coupling with the concentration detector also provides a useful automatic band broadening correction. This ensures that the correct concentration is assigned to the structure identified by the MS.

This type of GPC/SEC-MS coupling has been applied to a wide variety of macromolecules (homopolymers and copolymers); amongst them are polyacrylates, polystyres, polyethers, polyamides, resins, polycarbonates, proteins, and, polystyrene. Analysis goals have been synthesis optimization, investigation of the degradation behavior of polymers, polymer aging and deformation. We are sure that this new application field offers even more possibilities to gain detailed information on structure-property relationships.

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**PSS Contract Analysis**

**GPC/SEC - mass spectrometry – an important analysis tool to obtain true molecular weights for oligomers and REACH analysis**

The importance and the performance of GPC/SEC, particularly for the characterization of macromolecules, is well known. Separation is based on the size of the molecules in solution. Unfortunately GPC/SEC using only concentration detectors (UV/RID) is not an absolute method. Careful calibration of the system, to measure the average molecular weight and molecular weight distribution is required.

The calibration is usually done by measuring polymer standards, which are ideally chemically and structurally identical with the test sample. In this case true molecular weights are obtained. If no suitable standards are available and the true molar mass of the sample is required (e.g. for product registration), an absolute method needs to be applied. One such technique is GPC/SEC-light scattering, since this method does not require calibration with molar mass standards. However, a drawback of this method is the molar mass limitation. Reliable results with significant signal to noise ratio are only obtained for molecular weights > 5 000 Da. In addition, GPC/SEC-LS requires the knowledge of important sample parameters (e.g. the refractive index increment, dn/dc), which are not always accessible or constant.

**REACH Analysis**

A large group of technical products are oligomeric compounds. In these compounds, the first degrees of polymerization are still present. Here, the polymer status under REACH has to be demonstrated explicitly. To be classified as a “polymeric” material under REACH, fixed limits must not be exceeded. Based on a direct comparison with oligomeric calibration standards (e.g. oligomeric styrenes with defined degree of polymerization) the classical GPC/SEC analysis yields relative molar masses, which can deviate significantly from the actual “true” molar masses in the oligomeric product. Therefore the REACH criteria for a polymer can not be formally met in instances when the system was calibrated with inappropriate reference materials.

GC-MS analysis, which is a potential alternative method, can also be applied. However, here only the first oligomer fractions can be determined reliably. Thus, when applying this
method, the question is always whether the entire sample is really detected.

A solution for this dilemma is the coupling of GPC/SEC (or HPLC) with mass spectrometry. Especially hyphenation with ESI-MS is useful, since ESI-MS has its strength in the very interesting molar mass range from 4000 < M > 200. In addition, on-line coupling is easy. This is an additional advantage over other potential mass spectrometry methods, e.g. MALDI-TOF. Also ESI-MS does not require a matrix for ionization, that is required for MALDI-TOF.

ESI-MS generates line spectra. For each elution volume or oligomer the corresponding molar mass can be measured. Difficult, however, is a quantitative evaluation. This quantification is available after the evaluation of the elution concentration using a concentration detector (UV or RID) (see also the report on pages 2/3 of this streamliner). Therefore, in practice, the molar mass of individual oligomers can be measured in a direct, absolute calibration-free way.

Products, that can be analyzed by this method are:
- oligomeric resins (phenolic resins)
- isocyanate prepolymers (starting material for the production of polyurethane foams)
- oils (oxidatively treated soybean oils, linseed oil)
- fats and glycerides.

Also worth noting are polyester products generally used as lubricants.

A typical application is shown in Figures 1 and 2. Figure 1 shows the RI-chromatogram of an oligomeric phenol formaldehyde resin. Detected are signals for molecules with 1, 2 and 3 repetition units. Figure 2 shows the corresponding ESI mass spectrum for the degree of polymerization 1. Here three different co-eluting species that differ in the degree of substitution (2, 3 and 4 CH₂OH substituents) can be identified. Table 1 summarizes the possible and identified species for the different degrees of polymerization.

System Requirements:
Due to the ionization source design in ESI-MS measurement, there are several requirements for the mobile phase when the MS is coupled to an LC system. In general these requirements are the same as when working with an ELS detector:

The solvent should be easy to evaporate, only special solvent additives can be used. Eluents such as tetrahydrofuran (THF), toluene, chloroform and methylene chloride can be used without restriction. For aqueous GPC/SEC applications, where in most cases it is necessary to add a salt, it must be tested in each case whether a vaporizable salt (ammonium acetate) can be used.

Please contact our analytical department to discuss your analytical questions and to receive additional information.
Chromatographic Separations with online ESI-MS Detection

– Field study –

Advantages and applicability
One of the major advantages of electrospray ionization, ESI, is that there is a simple approach for coupling this ionization technique with chromatographic separations. Shortly after the introduction of ESI-MS as a new analytical method in the late nineties, online–SEC/ESI-MS applications were published for the analysis of polymers.[1] Since then, size exclusion chromatography (SEC) has proven to be a highly compatible separation method in online coupling with ESI-MS. The separation of the polymer molecules by size ensures that polymer fractions with a very narrow molecular weight distribution reach the electrospray ionization mass spectrometer, ESI-MS. The otherwise typical ESI-MS phenomenon, i.e. the mass spectrometric overlaps of different charged states due to a broad molecular weight distribution, is thus suppressed. Therefore, online coupling of SEC and ESI-MS generates easy to interpret mass spectra without overlapping masses from different charged states.

The sensitivity of the mass spectrometric analysis is significantly improved by SEC/ESI-MS coupling. This effect is based on the fact that only small fractions of the narrow distributed molecular weight distribution at a time are ionized, instead of the entire molecular weight distribution. Saturation effects have therefore less influence on the ionization efficiency. While samples in a molecular weight range of about 3 kDa can be analyzed by ESI-MS with direct infusion, the molar mass range after online connection can be extended to some 10-20 kDa (depending on the resolution of the mass spectrometer).[2] In addition, the preliminary separation removes low molecular weight impurities and matrix components that may otherwise interfere with the ionization process.

In contrast to biopolymers, most synthetic polymers cannot be ionized by formation of H+. Carbonyl, carboxyl and ether groups, however, have a high affinity for alkali metal ions, especially Na+, and to a lesser extent Li+, K+. As a rule of thumb, polymers bearing at least one of the above mentioned functional groups per monomer unit can be analyzed. Typical systems are poly(meth)acrylates, polyacrylamides, polystyres, polyamides, polycethylenes, polyesters, polyacrylonitriles, and another large number not specified above resin precursors and oligomers.

Technical implementation
Modern electrospray detectors are capable to handle eluent flows up to several 100 µl/min and can therefore be coupled easily with existing conventional HPLC equipment.[3] Hyphenation with µ-SEC columns have also been described (length 250 mm × 0.5 mm ID, flow rates 3-4µl).[4]

A suitable design of such an instrumentation used at the Karlsruhe Institute of Technology is shown in Figure 1.[5,6] The analytical HPLC system (Agilent 1200) consists of a pump, which pumps the eluent (THF) at a flow rate of 300 µl/min. The slight reduction in flow rate and the simultaneous reduction of the column inner diameter to 4.6 mm minimizes solvent consumption while maintaining separation efficiency. As columns high-resolution columns have proven to be the best choice, as the polydispersity of the incoming factions at the mass spectrometer can be minimized. Matching columns are PSS SDV linear 5 columns (1 x 4.6×30 mm + 2 x 4.6×250 mm, 3 µm particle size) that show excellent results for in-line coupling with ESI-MS. Smaller column dimensions have the advantage that the dilution effect is minimized.

Samples with an injection volume of 20 to a maximum of 100 µl and an analyze concentration of 3 to 10 mg/ml are typically applied to the system. Higher concentrations and injection volumes are especially needed for polymers with high molecular weight averages and a high polydispersity.

A parallel array of concentration-sensitive detectors (UV, RI, ELSD) and mass spectrometry, allows to operate all detectors under optimized flow rates. The bulk of the eluent (270 ml/min) is passed after the separation to the UV-Vis and RI detector to minimize broadening processes by mixing volumes of the detector cells, which are particularly significant at low flow rates. One-tenth of the eluent (30µl/min) is passed to the mass spectrometer. For this split ratio of 9:1, ideal tubing dimensions are shown in Figure 1.

Through a syringe pump, a methanol solution of sodium iodide (100 mmol/L) is added to the mass spectrometry eluent. The addition of defined amounts of sodium salt ensures reproducible and efficient ionization and allows suppressing any unwanted ionization in the solvent by existing cationic impurities (K+, Li+, NH4+, etc.). Although syringe pumps can be used, it is worthwhile investing in a piston pump (e.g. Teledyne ISCO, model DM 100) or a micro-flow HPLC pump to enable a reproducible and pulsation-free solvent flow. With dichloromethane (DCM) or tetrahydrofuran (THF) as eluent, a variety of hydrophobic polymers can be analyzed. However, it should be verified that fittings and tubing that have contact with DCM or THF should not be made from plastics such as PEEK or PTFE, since the solvents may lead to swelling of the material and the leaching of low molecular weight components. Although finger tight PEEK fittings have been used without problems in this system already for several years, special care has been taken to ensure that all connections and T-connectors were made of stainless steel. Recommended tubings are stainless steel, polyimide-coated silica and PEEKsil™.

Fig. 1

Table

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<thead>
<tr>
<th>Component</th>
<th>UV</th>
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<th>ELSD</th>
<th>Mass Spectrometer</th>
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Literature:

Author:
Dr. Till Gründling, Karlsruher Institute for Technology (KIT) Barner-Kowollik group
info@polymer.de
The introduction of UHPLC columns with small particle sizes (<2 micron) and small column dimensions has led to much faster analysis times and reduced eluent consumption within the world of HPLC. Seeking comparable advantages for SEC/GPC, has motivated PSS to develop new optimized materials with better resolution at shorter or comparable analysis times. PSS is therefore introducing herein the SUPREMA 5 µm columns, with unparalleled oligomeric resolution power for aqueous applications.

PSS development strategy accomplished two main objectives of achieving higher resolutions for low molecular weight separation, while separating high molar mass polymers (> 1 000 000 Da), fully aware that the UHPLC column model cannot be transferred directly into the GPC/SEC’s. There are physical limitations inherent to GPC/SEC, which separates large macromolecules with molecular weights reaching 10 Million Da, and hydrodynamic volumes from 10 nm to 500 nm.

- The first limitation to reducing the particle size in the GPC/SEC, is that large molecules have the potential to undergo shearing inside of columns exhibiting very low interstitial volume. Shearing is the break-up of the polymer chain as a result of stress on the chain.
- A second limitation refers to the size of the pores on the particles. Small particles especially with large pores have limited support matrices that decrease the stability of the particle.
- The higher packing density of smaller particles causes increased pressure inside the column.

New GPC/SEC Columns
New particle sizes for GPC/SEC

The resolution of a 5 µm particle size SUPREMA column is illustrated in Figure 1, compared to the resolution of a SUPREMA 10 µm column. Note that under the same conditions, a 5 µm 100 Å SUPREMA column is sufficient to separate Dextran into its oligomers, whereas, the SUPREMA 10 µm column shows only one symmetrical peak. This also illustrates the combination of two SUPREMA 5 µm 100 Å columns which yield a near-baseline separation in the low molar mass region. Figure 2 illustrates SUPREMA 100 Å 2 -column combination which allows the separation and identification of different polysaccharides. Notice the resolution of the sugars.

It was verified that the 5 µm particles do not affect the structure (shearing) of large macromolecules. High molar mass polysaccharides -Pullulan from 400 000 Da to 2 500 000- were measured using GPC/SEC coupled with multi angle light scattering. No sample degradation or molar mass changes were detected. With unparallel oligomer resolution, the SUPREMA 5 µm columns are available for a variety of aqueous applications with molecular masses ranging between 100 Da and 2 500 000 million Da.

For organic applications, PSS offers high-resolution SDV micro-columns. These materials can be used when better resolution at shorter or comparable analysis times is required or for the latest analytical LC-coupling methods, such as GPC/SEC-ESI-MS, which require coupling high-resolution columns with small total column volume (see also field study page 5).

The results presented here, first previewed at the 2010 column selection seminars, promoted lively discussions and interest among attendees. We would like to thank all for their contribution, and invite other column users to the next column meeting scheduled for November 8th 2011 in Mainz, Germany.

Fig. 1
Separation power of one SUPREMA 5µm (8 x 300 mm) compared to one SUPREMA 10 µm column for oligomeric Dextran (dxt T1 in water); plus the resolution gain from combining two columns SUPREMA (5 µm, 100 Å porosity)

Fig. 2
Separation and identification of various polysaccharides compared to T1 dxt. Measuring conditions: SUPREMA 5 µm 3x100 Å at room temperature and 1 ml / min in water.

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For our customers, the change means that we can continue in guaranteeing fast response times for delivery and support. In addition, they will profit from a pleasant working atmosphere at training courses that we conduct at our facility. As we have also expanded the capacity in the field demonstration laboratories, we can respond more quickly with instrument selection and help our customers to get a reliable picture of the performance of PSS systems.

What are your additional plans for 2011?

Now that we have the room available, we will train again new apprentices. After the very positive experience with Christian Wecker, who now works as a full PSS employee in order processing and seminar organization, the decision to continue with the youth education program was very easy.

In addition, we are continuing to strengthen our scientific team. The departments “GPC software and systems” and “column and particle technology” are looking for top-caliber personnel to join our staff.

In sales and support, we have just reinforced with Dr. Huub Bock, a well-known GPC/SEC expert. He has been working for about 1.5 years in close cooperation with PSS and is now a full-time employee at PSS GmbH Germany since January 2011. Mr. Bock will continue to take care of our customers in the Netherlands, Belgium and Luxembourg.

Also we have plans for our office in the U.S., necessary staff and infrastructure reinforcements are planned there.

2011 will be again an eventful and interesting year for PSS.
New PSS employee for the Benelux.

Since January 2011 Dr. Huub Bock is working for PSS.

He is responsible for supporting our customers in The Netherlands, Belgium and Luxemburg.

His office is based in The Netherlands.

Huub Bock has already been co-operating with PSS since mid 2009. He is a worldwide well known expert for GPC/SEC, Lightscattering (static and dynamic) and coupling techniques.

We are looking forward for an intensive co-operation!

Face-to-face training

**GPC/SEC Training**
- March 24. - 25. 2011
- September 22. - 23. 2011

**Hands-on Visco/LS**
- June 30. - July 01. 2011

**Software Training**
- WinGPC ReportDesigner plus: September 05. 2011
- WinGPC Basic Training: September 06. 2011
- WinGPC Viscometry/Light Scattering: September 07. 2011
- WinGPC SystemPilot: September 08. 2011
- WinGPC Compliance Pack: September 09. 2011

Single day booking available.

**Usermeetings**
- Column user meeting: November 08. 2011

All seminars in Mainz, Germany

Web-based training

**Webinars GPC/SEC Basic Training**
- April 20. - 21. 2011
- June 15. - 16. 2011
- August 17. - 18. 2011
- September 21. - 22. 2011

**Webinars WinGPC refresher**
- Basic WinGPC Refresher:
  - May 11. 2011; 11:00 AM EDT
  - September 14. 2011, 11:00 AM EDT
  - November 02. 2011, 11:00 AM EDT
- Advanced WinGPC Refresher:
  - May 12. 2011; 11:00 AM EDT
  - September 15. 2011, 11:00 AM EDT
  - November 03. 2011, 11:00 AM EDT

**The Viscometry, Light Scattering, Triple detection WinGPC Refresher:**
- May 13. 2011; 11:00 AM EDT
- September 16. 2011, 11:00 AM EDT
- November 04. 2011, 11:00 AM EDT

**Further PSS Webinars**
- GPC/SEC columns for protein characterization, April 06. 2011
- Copolymer characterization using liquid chromatography, April 13. 2011

Conferences/Trade Shows

**March 23. 2011**
Ecological GPC/SEC Seminar in Barcelona/Spain

**March 28. – 30. 2011**
241th ACS National Meeting & Exposition, Anaheim, California/USA, Booth 459

Advanced Polymer Characterization Workshop:
- March 28, 2011 8:30 AM - 11:00 ADT
  Room 211 B, Anaheim Convention Center
- April 26. – 29. 2011
  11th Annual UNESCO/IUPAC Workshop and Conference on Functional Polymeric Materials, Stellenbosch / ZA
  Booth and talk
  Peter Kilz: “Online Mass Spectrometry for Comprehensive GPC/SEC Characterization”
- June 26. – 29. 2011
  EPF 2011, Granada/E, Booth

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* Official language: English

PSS has representatives in the following countries:
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