Autumn 2007 A Joint publication of PSS GmbH, Germany and PSS USA

GPC Streamliner

Seeking Trustworthy GPC/SEC results?

Seek no more! Education, solutions, tools and tips, show once again PSS' solemn commitment to the success of Macromolecular Liquid Chromatography users. PSS offers services and off-the-shelf products that fulfill the national and international GPC directives, and empower our clients with dependable, reliable, accurate, compliant, and traceable GPC results; i.e., Trustworthy GPC/SEC .

PSS solutions for Trustworthy GPC/SEC support all aspects of GPC/SEC and all phases of the analysis from method development to daily operation in a regulated environment. This issue's ensuing solutions are:

- The new SECcurity GPC system which has a broad applicability with advanced detection (see page 1/2),
- semi-preparative capability (see page 5) and full support during all qualification steps, either with PSS tools and software (WinGPC Unity MCDS), or custom services.
- PSS high resolution GPC/SEC columns ensure interaction free chromatography, even for difficult samples (see page 4), under analytical, semi-micro and semi-preparative conditions.
- Solutions for non-destructive characterization of biopolymers and proteins with light scattering detection (see page 3).

Your success is our success. Contact PSS today for optimum personalized solutions. Or..., contact others for "One size fits all" solutions.

In This Issue

Advanced Detection with SECcurity

The PSS SECcurity GPC System supports diverse online detectors for all GPC/SEC methods and applications. Various detectors used simultaneously often provide supplementary results and evaluation options.

The SECcurity detectors are divided into two classes depending on their response to a sample, which may be described using the following simple equation:

$$SI = K_{Det} \bullet k_{sa} \bullet m_{inj} \bullet M^x$$

SI	Signal intensity
K_{Det}	detector constant
k_{sa}	sample dependent constant
m_{inj}	injected mass
M	molar mass

with

$x = 0$ for concentration detectors and

$x \neq 0$ for molar mass sensitive detectors.

SECcurity Concentration Detectors

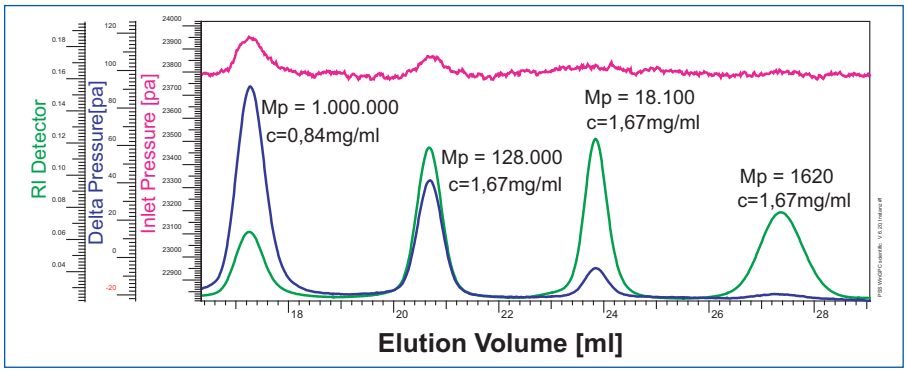
SECcurity, like other analytical GPC/SEC systems, operates with at least one concentration detector, e.g. an UV/DAD detector, a refractive index detector (RID), or an evaporative light scattering detector (ELSD). These concentration detectors are useful for

- Molar mass determination based on calibration
- Quantity measurement of residual monomer, solvent, or additive
- Copolymer composition or chemical heterogeneity (at least 2 concentration detector are required)
- Slice concentration measurement in combination with molar mass sensitive detectors
- 2-dimensional chromatography contour maps

» Continues on Page 2

Advanced Detection with SECcurity

» From Page 1



PSS SECcurity GPC System Polystyrene analysis (4 molecular weights) detected with Refractive Index (RI) and on-line viscometer ETA2010. The low molecular weight standard is easily detected even at the very low typical GPC/SEC concentrations. The nice and small peak shape of the viscometer signal (delta pressure) results from the high quality, fast-response Hastelloy pressure transducers.

The SECcurity concentration detectors boast exceptional features:

- Small cell volumes that prevent band broadening in the detector cell
- Fast solvent exchange
- Supreme sensitivity and stability
- Compatibility with GPC/SEC solvents including HFIP
- Ease-of-use with intelligent end-of-sequence actions
- Timely maintenance features for increased instrument uptime

SECcurity Molar Mass Sensitive Detectors

SECcurity supports the PSS GPC/SEC molar mass sensitive detectors: the on-line four-capillary viscometer ETA2010 and the on-line multi angle laser light scattering (MALLS) detector SLD7000. These detectors do not require matching calibration standards to provide molar mass, and they can be used for structure and branching investigation.

The combination of the two detectors (to the concentration detector(s)) establish Triple *plus* detection to the system.

The following distinctive features compliment the SECcurity molar mass sensitive detectors:

Viscometer ETA2010:

- Asymmetric bridge 80/20: only 20 % of the sample is diluted in the solvent path, 80 % of the sample is left to be detected in the sample path
- Solvent-resistant, long-life, and fast-responding Hastelloy pressure transducers
- Automatic pressure transducer protection system for secure operation
- Peak sensitivity even at low concentrations and molar masses

MALLS detector SLD7000:

- Simultaneous measurement of 7 angles (35° - 145°)
- Cylindrical cell with index matching results in low numerical aperture and solvent independent scattering angle
- Small cell volume
- Intelligent vertical cell construction prevents trapped particle or air bubbles scattering interference and tedious cell cleaning

Additionally the SECcurity GPC system can be coupled to the FTIR/MALDI-interface for sample identification.

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Announcements

New staff in Mainz

Dr. Jürgen Paulsdorf joined our software and GPC instrument team on 01.02.2007. He is a chemist with five years of experience in the field analytical application. You may contact him regarding GPC equipment, molar mass sensitive detectors and GPC software.

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www.polymer.de Makeover

PSS brand new website will debut late September, 2007. Easier navigation and powerful search functions will bring forth detailed information about PSS and its comprehensive macromolecular characterization product line by Gel Permeation Chromatography:

- Liquid chromatography (LC GPC) hardware and software solutions
- Advanced GPC column technology and separation techniques
- The broadest range of macromolecular reference polymers in the market
- Contract analysis for macromolecules
- Services, documentation, applications, support information and training.

JPMorgan Chase Corporate Challenge

Frankfurt, 13. June 2007, a team of seven PSS men and women run the JPMorgan Chase race. This annual event challenges worldwide workers participation to raise funds that benefit local charities (> $1.5 million in the last three years), while enjoying a creative outlet for exercise and camaraderie.

Study of protein denaturation by GPC/SEC - MALLS

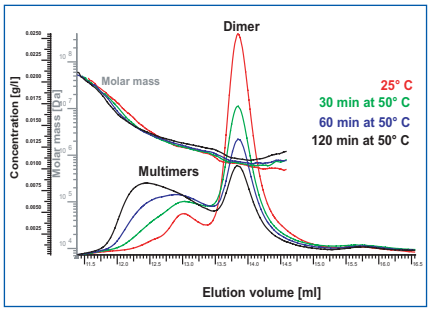

Fig. 1: Hydrodynamic volume change and resulting molar mass of bovine Thyroglobulin as a function of temperature Concentration detector signal). Red curve is the native protein including the associates

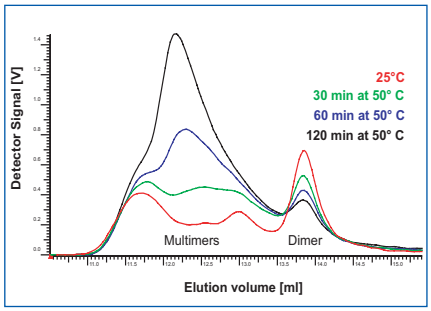

Fig. 2: Denaturation of Thyroglobulin (bovine) as a function of temperature, shown: 90° MALLS detector signal. The red curve is the native protein including the associates.

The combination of GPC/GFC/SEC separation with light scattering detection is a powerful method to study the structural transition of proteins. GPC/SEC separates (by size) the proteins into monomers and higher aggregates, while the on-line MALLS detector measures the molar mass and structure simultaneously.

Proteins in the native state are folded into unique 3-dimensional structures, stabilized by hydrophobic and electrostatic interactions, as well as by hydrogen bonds between the structural amino acids side chains. Altering environmental parameters such as pH or temperature triggers entropically driven protein denaturation processes, i.e., the transition of the native 3-dimensional structures into random coil structures. During this process the secondary and tertiary structures are destroyed. The structure of a protein determines its function; therefore, the ability to monitor structure is very valuable.

Experimental

Bovine Thyroglobulin, M = 670.000 Da., (Sigma) was exposed to 50 °C temperature for periods of 30, 60 and 120 min. The structural changes were measured using a SECcurity GPC1200 system, equipped with RI, UV and PSS SLD7000 MALLS detectors, and WinGPC Unity software (with MALLS module) for data acquisition and processing. The molecular size separation was achieved with a combination of columns, PSS PROTEEMA 5 µm, 100 Å + 300 Å (each 8 x 300 mm). Samples were eluted in phosphate buffer (34 mmol/l) at pH 6.6 with NaCl 0.5 mol/l at the following conditions:

flow rate:	1 ml/min
injection volume:	20 µl
sample concentration:	1 g/l
temperature:	25 °C

The results are presented in Figures 1 and 2.

Results

The molar mass vs. elution volume behaviour for the various heated samples stays constant during the transition, because only the structure is changing. This is noticeable in the molar mass dependency of Figure 1. However, the dimer signal decreases, and shows a shift to lower elution volumes, which indicates the transition from a very compact, globular structure to a lesser dense (entropically driven) random coil structure. The transition occurs within the first 30 minutes exposure of the protein solution to 50° C. After 120 minutes, the native monomeric chains suffer more than 50 % denaturation. Native monomers and associates tend to denature simultaneously.

The general increase of the molar mass at smaller elution volume, regardless of the structure, is due to the association or agglomeration of the protein.
The 90° light scattering signal presented in Figure 2, confirms a decrease of the native dimer and the increase of the denatured associated structure as a function of temperature.

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Innovations

PSS WinGPC Unity Premium Support Contract

The PSS software department offers a software support contract for all WinGPC users.

Profit from the following rewards:

- Priority telephone, fax, and e-mail support
- Guaranteed response time within 24 hours
- Free WinGPC patches
- Free WinGPC updates
- 20 % reduced training fee for WinGPC software trainings and PSS GPC/SEC courses
- NetViewer teleconference (online Software-Support) free of charge

Part number: 899-0009

Membrane Analysis Standards

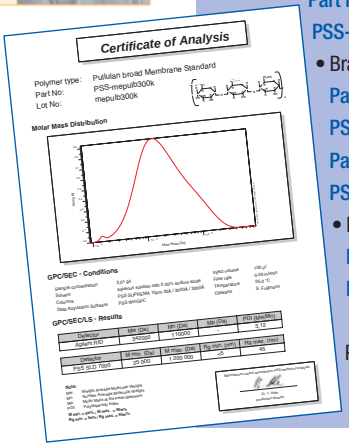
PSS has made available three lines of reference standards for the fast and robust GPC/SEC characterization of membranes in their native environment (swelled state). These reference standards can be used to prepare stock solutions for filtering experiments as e.g. described in an article of P. Kilz, M. Viktorin in LCGC Europe April 2007:
http://www.lcgceurope.com/lcgceurope/article/articleDetail.jsp?id=414754&pageID=1&sk=&date=

The membrane standards feature the following properties:

- Broad Molar Mass Distribution for all types of membranes with different pore sizes
- Detailed product certificate of analysis with
  Molar Mass Averages M_w and M_n
  Integral Molar Mass Information M_{min}, M_{max}
  Corresponding Radii of Gyration $R_{g,min}$ and $R_{g,max}$.

Order information:

- Linear Polysaccharide (Pullulan):
  Part number:
  PSS-mepulb300k (M_w: 300 000 Da)
- Branched Polysaccharide (Dextran):
  Part number:
  PSS-medxtb70k (M_w: 70 000 Da);
  Part number:
  PSS-medxtb2m (M_w: 2 000 000 Da)
- Polystyrene:
  Part number:
  PSS-mepsb200k (M_w: 200 000 Da)



Packing size: 50 g,100 g, 250 g, 500 g.

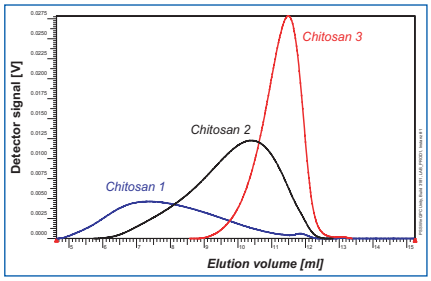
Interaction-free GPC/SEC for Polycations

PSS NOVEMA columns are successfully established for the GPC/SEC of polycations, like Cyclodextrins and aminated Chitosans, Poly(2-vinyl pyridine) and PolyDADMAC, boasting advantageous when compared to other columns:

- OH-functionalized methacrylate copolymer network, which has a neutral surface
- Facilitates method development; the neutral surface eradicates dependency on the functional group density of the polycation
- One solvent system can be used for the majority of polycations

Polycations, are among the most difficult samples to evaluate in GPC/SEC because they present strong chemical interactions with the traditional methacrylate columns materials generally used. Even the addition of salt to neutralize the surface does not usually help to overcome this limitation.

Recognizing the importance of the polycations product class, PSS designed a stationary phase specifically for their characterization. The two options detailed in Table 1 were considered as development strategies: Option 1, to produce a cationic-modified charged surface that neutralizes the attractive interactions; or Option 2, to produce a new material with a neutral surface that inherently prevents attractive and repulsive interactions. The Option 2 generated a stable, resistant and robust polymeric stationary phase that overcomes the interactions of polycations during GPC/SEC, i.e., PSS NOVEMA.



Overlay of three different Chitosan samples, differing in molar mass and molar mass distribution. Conditions: Eluent: H_2O, 0.1 M NaCl, 0.1 % TFA; Columns: PSS NOVEMA 10 µ, 30 Å+ 1 000 Å + 1 000 Å (each 300 x 8 mm), precolumn.

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Comparison of polycation stationary phase development strategies

	Option 1	Option 2
Strategy	Cationic modification of existing GPC/SEC stationary phase	Create a new stationary phase with neutral surface specifically for polycations
Interactions	Prevents attractive but adds repulsive interactions	"Non charged" surface prevents attractive and repulsive interactions
Rewards	• Easy stationary phase development • No salt-addition required	• Prevents all types of interaction • Better agreement with GPC/SEC separation requirements • Fits universal calibration conditions • Easy comparison with neutral polymers or polycations with lower functional group density • Easy method development • One solvent system for most polycations • Maximum accessible pore volume available • High resolution due to optimized pore volume
Drawbacks	• Needs organic modifiers and organic acids • Adds repulsive interaction • Invalidates universal calibration (neutral and charged polymers see different pore sizes) • Reduces accessible pore volume, ower exclusion limit • Polyanions may stick to the surface and damage the column	• Enhanced development effort for new stationary phase • Requires addition of salt and organic acids
application examples	Cyclodextrin: 75/20/5 H_2O, MeOH, AcH* Aminated Chitosan: 95/5 H_2O, AcH*	Cyclodextrin: H_2O, 0.1 M NaCl, 0.1 % TFA** Aminated Chitosan: H_2O, 0.1 M NaCl, 0.1 % TFA** Chitosan (see figure 1): H_2O, 0.1 M NaCl, 0.1 % TFA** Poly(2-vinyl pyridine): H_2O, 0.1 M NaCl, 0.1 % TFA** Poly(DADMAC): H_2O, 0.1 M NaCl, 0.1 % TFA**

* Viscotek application on VicoGel PolyCat columns
** PSS application on NOVEMA columns; further applications: www.polymer.de, Knowledge bank.

Upcoming events

GPC training courses in Mainz, Germany:

11. – 12.10.2007

28. – 29.02.2008

09. – 10.10.2008

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.

Shows and Exhibits

09.09. – 11.09.2007
Bayreuth Polymer Symposium; Bayreuth, Germany

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
Please visit our booth

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Semi-preparative GPC/SEC

Call on PSS to evaluate and supply your specific need for semi-preparative GPC/SEC including suitable preparative columns. The new analytical PSS SECcurity GPC/SEC system will perform semi-preparative fractionation for you or PSS will adapt existing HPLC components to achieve your goal. GPC/SEC is an established analytical method for sample fractionation and analysis of molecular weights in quality control and research laboratories. There are many applications which only require small-scale (few grams) sample fractionation, for example, for enrichment/cleaning of samples during organic synthesis or preparation of samples for supplementary analytical investigations, e.g. infrared spectroscopy, NMR, mass spectrometry or elementary analysis. These requests can be effectively handled with a semi-preparative GPC/SEC system in a straightforward manner.

Fig. 1: PSS SECcurity GPC/SEC system with manual injector and fraction collector (courtesy of H.Thijs, Eindhoven University of Technology).

A basic GPC system like the PSS SECcurity requires relatively small investment to become a powerful semi-preparative GPC/SEC instrument: 1) preparative GPC/SEC columns, 2) the ability to inject larger sample amounts; 3) an automatic fraction collector to simplify the fractionation process; 4) adequate control.

PSS' typical preparative GPC/SEC columns (300 mm long x 20 mm ID) will work at a flow rate of around 6.25 ml, which can be achieved by most analytical HPLC instruments. Due to the unique design of PSS columns, separation methods developed with analytical columns are easily transferred to semi-preparative scale. This has the advantage that whenever the detector limits the flow to analytical conditions (1.5 ml/min) the semi-preparative separation can be carried out bypassing the detector.
PSS wide range of preparative GPC columns are described in the consumables catalogue *(PSS Reference Materials & LC Columns)* and website.

Larger injection volumes (between 1 and 2 ml) are obtained installing a sample loop to the manual injector or upgrading the autosampler with the semi-prep upgrade kit.

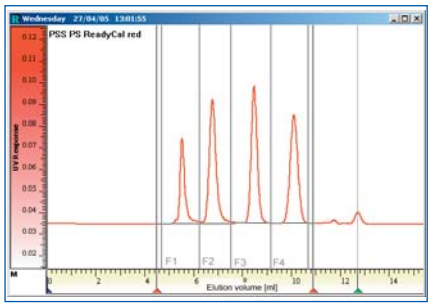

Fig. 2: WinGPC raw data with fraction marks

The powerful fraction collector, PSS model 122 SB, flexibly adapts to a fractionation strategy (heart-cut, peak recognition, time, volume, etc.) and size of the collection vessels suitable to the specific application.

The fraction collector is easily controlled by the WinGPC Unity software and the intelligent UDC810 interface. Every time the fraction collector moves to the next storage vessel a fraction marker is inserted into the chromatogram to identify the fractions in the sample (see Fig. 2). The complete fractionation of a sample can be achieved unattended, within hours, with repetitive automatic injections.

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Please visit our booth

A91 in hall 1.1
at ILMAC 2007
in Basel, Switzerland
25.09. - 28.09.2005

Application

Characterization of Epoxy Resins

Epoxy resins are typically oligomeric compounds containing more than one epoxy function. Many Epoxy resins are prepared by the reaction of bisphenol A with epichlorhydrin. They are used for the preparation of duroplasts by polyaddition with polyfunctional amines (hardeners) or carbonic acids/anhydrides. Typically they are used in electronic industry for isolation, as coatings, glue for plastics or for sealing.

Sample preparation:

The sample was dissolved for 12 hours. The sample solution was filtered through a 0.45 µm membrane filter to protect the columns.

Analytical conditions:

Eluent:	THF
Columns:	PSS SDV 5 µm 100 Å + 1 000 Å + 10 000 Å (each 8 x 300mm) + precolumn
Calibration kit:	PSS ReadyCal Poly(styrene) low (Mp: 266 - 67 500 g/mol)
Data acquisition:	PSS WinGPC Unity
Detector:	SECcurity GPC1200 RI
Flow rate:	1.0 ml/min
Sample Concentration:	5.0 g/l
Injection volume:	20 µl
Temperature:	25° C

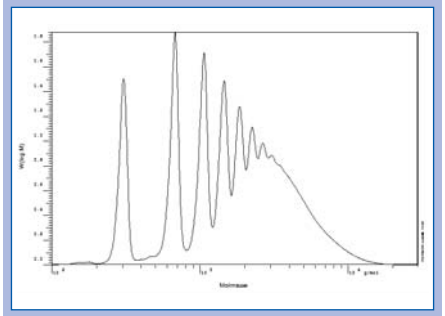

Figure 1: Molar mass distribution of an epoxy resin sample

Results & Conclusion:

The combination of SDV columns (above) separates the resin oligomers up to P8, at the analytical conditions given above. Characterization of epoxy resins require columns with high resolution in the low molecular weight area, where the oligomeric distribution of the sample elutes.

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