Edition spring 2012

GPC STREAMLINER

GPC/SEC Solutions and Expert Support

GPC/SEC/GFC is a versatile technique for characterizing polymers, biopolymers, polysaccharides and proteins. And GPC/SEC is our passion!

We at PSS are fully dedicated to the advancement of macromolecular liquid chromatography by means of developing true solutions and providing competent and personal support. Based on excellent products and latest findings in material science, we create easy-to-use and powerful solutions for QC and R&D. From a single molar mass reference material to turn-key systems for GPC/SEC multi detection with light scattering, viscometry, mass spectrometry or fully compliant GPC/SEC for the pharmaceutical industry: PSS offers all products and services for successful macromolecular analysis and expert support by GPC/SEC enthusiasts.

In 2012 we present our new solutions at several trade shows worldwide. You can meet PSS at Pittcon, Analytica, and Achema as well as on several smaller meetings and conferences.

At these events we introduce:

Metal-free BioSECcurity SEC System:
Our new multi detector solution for the absolute analysis of biopolymers under harsh conditions

WinGPC UniChrom GPC/SEC MCDS software:
When multi detection light scattering and viscometry is not enough: 2D chromatography and mass spectrometry as powerful hyphenated techniques

Viscometry/Light Scattering GPC-Addon for Agilent OpenLab ChemStation:
Add new functionality to your standard environment for acquisition, evaluation, reporting

HT Contract Analysis:
New temperature gradient HPLC method for polyolefin separation

Other solutions, products, and services can be found on our website www.pss-polymer.com and in our new brochure GPC/SEC solutions.

Other chances to see our products in action and to talk to our developers are during one of our trainings or webinars. All dates for 2012 can be found on our website, the next events are listed on page 8 of this Streamliner.

Yours

In this issue

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System Solutions

BioSECcurity, the metal-free GPC/SEC solution with light scattering and/or viscometry

Routine bioanalysis under harsh conditions has never been so easy and powerful. With the BioSECcurity SEC System, PSS introduces the ideal solution for protein molar mass, purity, and aggregation analysis with advanced detection.

Problems in biopolymer analysis are often caused by corrosion due to the required high salt concentrations and the extreme pH values. In addition, metal ions can lead to problems with the column lifetime or chromatographic artifacts.

BioSECcurity overcomes these problems by offering a metal-free system comprising:

- a quaternary pump with active seal wash
- a high performance autosampler for well-plates or vials or alternatively a manual injector
- a multi wavelength UV detector or DAD/PDA
- a right angle multichrom light scattering detector SLD1000B (RALLS) or alternatively a multi angle light scattering detector SLD7000B (MALLS)
- an ETA2010B online viscometer
- an analytical fraction collector for well-plates, test tubes, or vials

The two light scattering detectors offered differ in the number of supported angles. While the RALLS detector measures the scattered light at 90°, the MALLS detector measures the scattering intensity simultaneously at 7 angles between 35° and 145°.

For many porteins, especially globular proteins, the RALLS detector is sufficient. The major advantage of this detector over others

is the user selectable wavelength for highest sensitivity even at low molar masses or concentrations.

The MALLS detector has to be used to determine the molar mass of anisotropic scatterers correctly or if the radius of gyration needs to be measured.

Wetted materials in this system are PEEK, PEKK, PTFE, Titanium, Gold, Platin-Iridium and Sapphire. Depending on the configuration the system can withstand pH values from 1-13 (1 day: 14) or 1-12 if the RALLS detector is used.

The following columns with PEEK hardware are available:

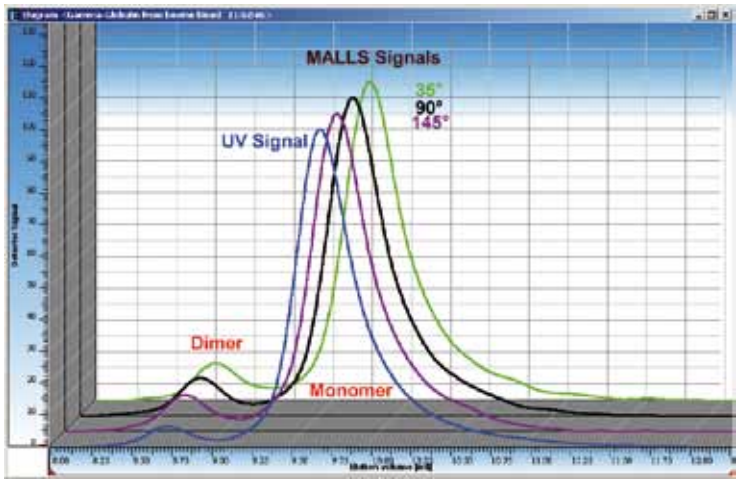
- PROTEEMA Precolumn 8*50
- PROTEEMA with different particle sizes and the porosities 100 Å, 300 Å and 1000Å.

Other materials on request.

Instrument control, data acquisition and analysis is achieved with WinGPC UniChrom MCDS. More details on UniChrom can be found at page 3.

Ideal applications for the system are protein molar mass, purity, and aggregation analysis. Here the addition of a light scattering detector adds new possibilities. This molar mass sensitive detector allows the determination of absolute molar masses without column calibration. Aggregates can be detected with superior sensitivity as shown in figure 1.

Figure 1



Gamma-Globulin sample (monomer with dimer) detected using a UV detector and the 7 angle MALLS detector (4 angles not displayed)

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GPC/SEC Software

New in WinGPC UniChrom GPC/SEC MCDS software

WinGPC UniChrom is a macromolecular chromatography data system (MCDS) supporting all methods in (bio)polymer characterization with controls for many vendors. It is the successor of the well-known WinGPC Unity software and comes with all Unity features and modules as well as many new options and functions. The most important new developments are shortly introduced in this article:

- The ChromPilot for comprehensive instrument control and powerful sequence management
- The Project Manager for modern sample management and fast access to data with less mouse clicks
- The Mass Spectrometry Modul for the analysis of GPC/SEC-MS data and determination of absolute molar masses for low molar mass samples

ChromPilot

With the release of WinGPC UniChrom and ChromPilot in November 2011, PSS has a system at hand that can easily incorperate controls for LC systems independent of brand. The flexible driver concept allows the fast and seamless addition of new systems eliminating the need to re-install and re-configure WinGPC UniChrom, when control for another system is added.

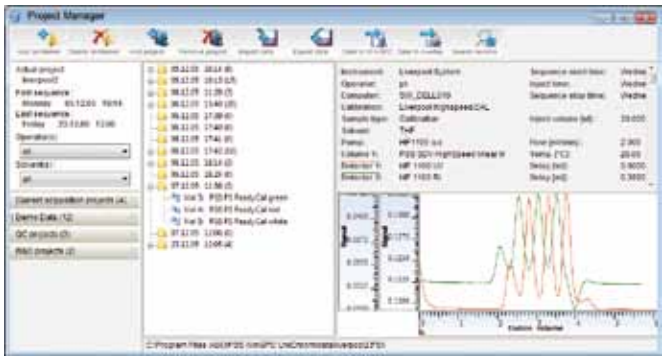
Figure 1

The ChromPilot for comprehensive system control for many vendors. The concept is driver based, new drivers March 2012: Waters Alliance and PSS BioSECcurity SEC System.

In March 2012 the following systems and detectors are supported by ChromPilot:

- PSS SECcurity GPC System
- PSS BioSECcurity SEC System (New March 2012)

Figure 2



The new WinGPC UniChrom Project Manager with the containers saves mouse clicks and offers a data preview.

- HP/Agilent 1100 System
- Agilent 1200/1260/1290 System
- Agilent 1120/1220 System
- Dionex UHPLC+ Focused, UltiMate 3000
- Shimadzu Nexera, Prominence, LC-8/10/20A
- Shodex RI 100/200
- Tosoh EcoSEC semi-micro GPC System
- Waters Alliance (New March 2012)
- Wyatt DAWN DSP, DAWN EOS, Mini-Dawn

New drivers are constantly developed and added.

The ChromPilot offers a number of advantages:

- Users can select the best instrument and the best software solution for their needs and still be sure that handling errors are avoided and compliance issues are met.
- Mixed configurations, e.g. pump, autosampler, and detectors from different vendors, are fully supported.
- The sequence manager and user interface are always the same, independent on the system used. This minimizes the requirement for training and makes GPC/SEC easy-to-use.
- The sequence manager does not only allow to program injections. It reflects the workflow in the lab by adding sequence commands that allow to change the flow-rate, the UV detector wavelength, the instrument or WinGPC method and other parameters during the sequence.
- The instrument manager shows a concise and clear summary of the most important parameters for all modules and allows to set comfortable end-actions.

Therefore the ChromPilot is an indespensable tool making lab life easier.

UniChrom Project Manager

The amount of data in labs grows continuously and directory trees, especially in networks, get more convoluted. That makes data management complex and more tedious. The WinGPC sample search feature is already a big help to find relevant information. A more universal tool is the new WinGPC UniChrom Project Manager. Projects can be organized in user defined containers, which hold WinGPC projects independent of their file location. So various WinGPC projects can be organized without the need to remember the file and network path.

Very useful is the comprehensive chromatogram preview and the filters which allow to show only a subset of sequences as specified in the filter definition. Data can be send directly from the Project Manager to the overlay which allows to create overlays quickly. There is no need to load any projects or sequences.

Mass Spectrometry (MS) Module

Light scattering is an accepted method to measure true molar masses. However, it has its limitations for low molar masses and for copolymers. Therefore, hyphenation of GPC/SEC with mass spectrometry (ESI-MS, MALDI-

Is WinGPC UniChrom right for you?

Arrange a personal web meeting with one of our experts. Based on your application requests and personal questions, we will show you features and functions that make your life easier!

Contact info@polymer.de

ToF) has become a new, valuable tool to measure true molar masses with high precision, that cannot be determined with any other method.

The disadvantage of GPC/SEC-MS hyphenation, the complicated analysis especially when copolymers are present or when multiple charges per chain are possible, has been overcome with the development of the UniChrom Mass Spectrometry Module. Here, mass spectra (in the vendor independent mzXML format) can be imported to the corresponding WinGPC run. The spectra are analyzed automatically and require only the molar mass of the repetition unit (2 values in case of copolymers) and the molar mass of the ionization agent as user input. Isotope effects in the MS spectra are automatically corrected, the charge state in ESI spectra is automatically determined without any user interaction. This makes things easy also for non MS experts.

The usability of the WinGPC MS module is very similar to the light scattering, viscometry and 2D modules and can be mastered without much effort or long-time training. The data analysis offers many features, depending on the MS technology employed. These include structure elucidation with MSn, ion-trap and MS-TOF instruments. Even "simple" MS (single quads) generate very accurate molar masses which makes them very useful for absolute molar mass determination without the need to know dn/dc or avoiding copolymers as in light scattering, triple or tetra detection.

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GPC/SEC Software

PSS GPC/SEC Multi-Detector Software for Agilent ChemStation

The PSS WinGPC product family and Agilent's ChemStation are very popular and widely used chromatography data systems for GPC/SEC and LC, respectively. A GPC/SEC addon for the ChemStation CDS, targeted for routine applications and limited to concentration detectors, is available. However, with the availability of light scattering (LS) and viscometry (DV) detectors in the Agilent product line after the acquisition of Polymer Labs/ Varian, there is a need for a more feature-rich ChemStation GPC/SEC addon, that also supports molar mass sensitive detectors.

Based on the existing solution PSS took the lead and developed a fully integrated GPC/ SEC addon which supports concentration detectors, every kind of LS (RALLS, TALLS, MALLS) detectors and online viscometry. In addition, the PSS Multi-Detector GPC/SEC addon offers all kinds of calibration and evaluation methods incl. narrow and broad standards, universal calibration, viscometry, light scattering and triple detection.

Figure 1

OpenLab CDS ChemStation with activated PSS GPC/SEC Multi-Detector Addon showing GPC/SEC data from an MDS390 with 2-angle LS and viscometry

Figure 2

GPC/SEC data evalation settings support up to 6 signals and conventional, LS, DV and triple detection systems

Supported systems

Agilent	all 11x0,12x0 systems and modules, MDS390
PSS	all SECcurity systems and detectors
other	via Agilent
detectors	35900E A/D converter

Supported ChemStation revisions

ChemStation	all 32bit (B.01.01 to B.04.03)
OpenLab	all CDS CStn (C.01.0x)

Supported 32/64 bit Windows platforms

Windows XP, Vista, Windows 7,
Windows 2008 R2 Server

Instrument control, sequence programming, data evaluation, GPC/SEC settings, etc., is all done within the known ChemStation user interface and data formats. ChemStation methods, baselines, peaks and GPC data evaluation parameters are used by the GPC/SEC Multi-Detector Addon to calculate GPC/SEC results based on the ChemStation method.

The PSS GPC/SEC Multi-Detector Addon adds a GPC/SEC menu to the ChemStation data analysis view as shown in **Figure 1**. Automated full or partial sequences, batch analysis and interactive data analysis is supported. IQ and OQ of the software can be done easily by users with provided verification schemes. The GPC/SEC data analysis method supports all data analysis methods as can be seen in the dialog depicted in **Figure 2**.

The signal type directs data analysis for conventional (RI, UV), light scattering (7°, 15°, 90°), viscometry (DP, IP) and triple detection data processing.

Special data analysis methods like "Multipeak Analysis" and "Dextran Evaluation" for Pharmacopeia compliance are also supported.

The setup of detector combinations is made easy with a detector setup wizard, which automatically determines inter detector delays, calibrates concentration detectors, determines LS instrument constants, measurers LS normalization coefficients, etc.

GPC/SEC settings are part of the ChemStation method. Data processing can be either issued from the GPC/SEC menu ("calculate GPC results") or directly from the ChemStation sequence. **Figure 3** shows the interactive screen of a MDS390 triple detector data file with a VWD as concentration detector for a cocktail of narrow calibration standards.

Results can be printed on screen, to PDF, to a physical printer and can also be transferred to a LIMS or other result management system.

The PSS GPC/SEC Multi-Detector Addon has a Report Designer option which allows to create user defined result layouts.

Upgrade recommendations for existing installations

Users which already use either system have more choices now, their previous investment is always safe and maintained. The table indicates our upgrade recommendation for users which already have an existing data system and want to process GPC/SEC data with concentration and/or molar mass sensitive detectors.

Table 1

Current Data System	New detector(s) or method	Upgrade recommendation
ChemStation	concentration (e.g. RI, ELS)	PSS GPC/SEC Addon
ChemStation	light scattering (e.g. MDS390, PSS SLD1000)	PSS GPC/SEC MD Addon
ChemStation	viscometry (e.g. MDS390, PSS eta2010)	PSS GPC/SEC MD Addon
ChemStation	triple detector (e.g. MDS390, PSS Triple^{plus})	PSS GPC/SEC MD Addon
WinGPC	concentration (all vendors supported)	PSS WinGPC UniChrom
WinGPC	light scattering (all vendors supported)	PSS WinGPC UniChrom
WinGPC	viscometry (all vendors supported)	PSS WinGPC UniChrom
WinGPC	triple detector (all vendors supported)	PSS WinGPC UniChrom

Upgrade recommendations for existing installations

The advantages of using the PSS GPC/SEC Addon

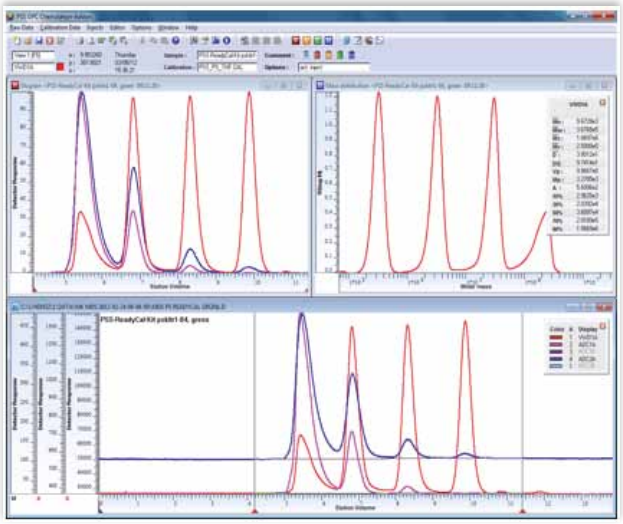
- uses well-known ChemStation software environment
- integrates seamlessly into ChemStation/ChemStation OL CDS
- same look & feel
- based on established and well supported product
- only incremental software training
- PSS method and Agilent instrument support
- highest functionality and flexibility
- keep what you have - add what you need

Since the PSS GPC/SEC MD Addon, as the Agilent GPC Addon, is based on the ChemStation architecture, data structures and principles, there are some limitations, which are not present in PSS WinGPC Unity or UniChrom:

- no database features available as ChemStation uses a file-based data concept
- no true 21CFR11 compliance
- no 2-dimensional data analysis
- no GPC/SEC-MS data analysis
- no Heparin (end group) analysis

In such cases the PSS WinGPC UniChrom software is the natural upgrade path which requires minimal user training to overcome Addon limitations. PSS WinGPC UniChrom can read all ChemStation data files which ensures that existing data can be reviewed and re-analyzed if required.

Figure 3



Interactive data analysis and result screen of a calibration mixture run on an Agilent MDS390 triple detector system with LS (pink), viscometry (blue) and UV (red)

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Contract Analysis

New temperature gradient HT-HPLC method for polyolefin separation

Many polyolefins differ not only in their molecular weight distribution, but also in their composition (PP, PE-olefin copolymers, PE) and morphology (HDPE, LDPE, LLDPE).

The molecular weight distribution can be determined by GPC/SEC. However, since GPC/SEC separates according to hydrodynamic volume and not to molecular weight, polyolefin blends of different composition may not be separated. Advanced detection techniques, e.g. light scattering or viscometry, cannot overcome this limitation, since they rely on proper separation.

One potential solution is the use of a separation methods based on the crystallinity (e.g. TREF, CRYSTAF, CEF). Another possibility is to use gradient HPLC, as a method that separates according to chemical composition based on the interaction between analyte and column material. Method development includes optimizing solvent composition or temperature.

Due to their limited solubility polyolefins have to be investigated at high temperatures (> 100°C), preferably in solvents such as TCB (1,2,4-trichlorobenzene). Only a few HPLC columns are available that can be used with these conditions. For the new PSS method a graphite based hypercarb column was used. In addition to a high temperature resistance, this column shows unique separation power. Due to the special surface chemistry separation depends not only on the composition of the sample, but also on the planarity of the molecules. Applied to polyolefins, this means that the separation depends primarily on the length of the ethene sequences.

In high temperature-temperature-gradient-interaction-chromatography (ht-TGIC), the sample is injected at higher temperature and then cooled down rapidly. This procedure leads to the accumulation of the ethene segments on the graphite surface. When the temperature is slowly increased, these segments resolve in accordance to their length: short segments elute first at lower temperatures, longer segments elute at higher temperatures.

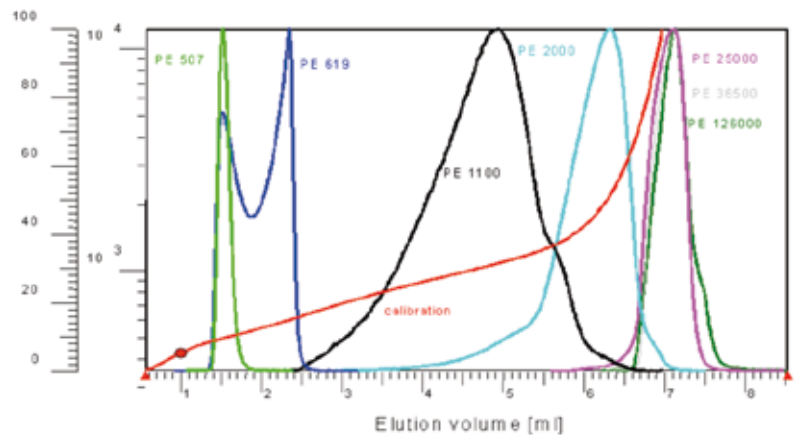
Figure 1 shows several linear polyethylenes with different molecular weights elute at different volumes/temperatures. Up to a molar mass of about 500 g/mol, the separation is based on the hydrodynamic volume (GPC/SEC mode). Between 500 to about 2000 g/mol, a linear relation is observed, where the elution volume depends on the length of the ethene sequences. Above 20 000 g/mol all chain lengths elute at approximately the same volume. A separation is not possible any more. The separation behavior is visualized by the red calibration curve in Figure 1.

The unique strength of ht-TGIC is shown for the separation of structurally different polyolefins. Here an infrared detector can be used, since isocratic conditions (eluent TCB) are applied. This allows to determine the concentration as well as the amount of CH_3-groups. In this example the CH_3/CH_{total} ratio was evaluated. It varied from 0.53 for linear PE to 1.18 for PP (33.3% CH_3 groups).

Figure 2 shows the different behavior of polyolefins in ht-TGIC.

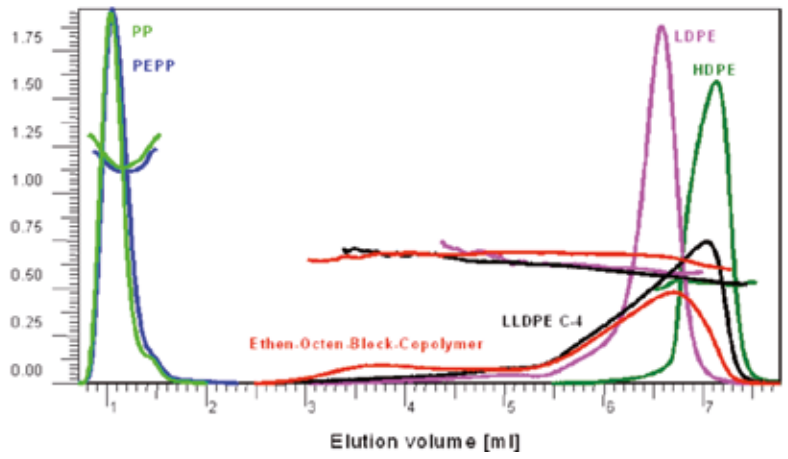
- Pure PP has eluted in approximately 1 ml in pure GPC/SEC mode (no interaction).
- A PE-PP copolymer with low PE content behaves similarly, as there are no long PE segments. Based on the CH_3/CH_{total} ratio it is possible to distinguish between both materials.
- HDPE elutes late, because of the long ethene segments which are adsorbed on the graphite surface. The measured CH_3/CH_{total} ratio of 0.53 hereby shows no dependence on the separation (constant composition).
- Due to the presence of long chain branching, LDPE has shorter ethene segment lengths than HDPE. This is reflected by elution at lower volumes. A higher amount of long chain branching results in earlier elution of LDPE. This is also confirmed by the observation that the CH_3/CH_{total} ratio decreases with the elution volume.

Figure 1



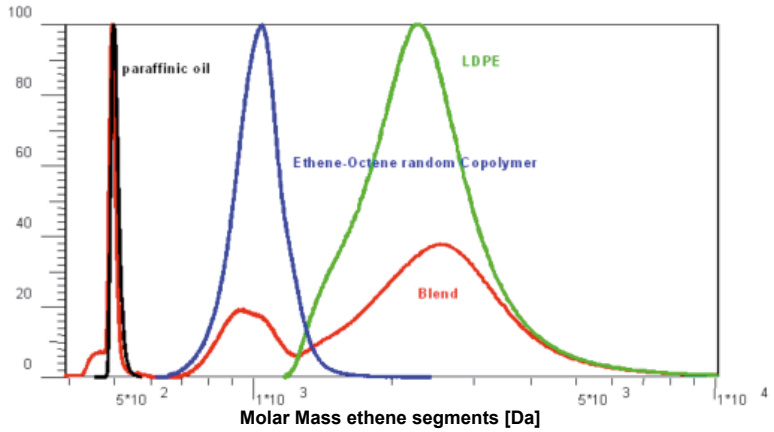
ht-TGIC linear PEs of different molecular weight, calibration based on ethene sequence lengths

Figure 2



ht-TGIC for different types of polyolefins

Figure 3



ht-TGIC ethene sequence distribution for a blend and its components (normalized to heigth)

- A statistical LLDPE (ethylene-butene copolymer) shows a different elution behavior. The random incorporation of long ethene sequences leads to an elution at almost the same volume as HDPE. With the incorperation of butene, the ethene sequence length, and thus the elution time, decreases. This continuous change is reflected directly in the CH_3/CH_{total} ratio, which decreases almost linear with increasing elution volume.
- Compared to a random copolymer the elution of a block copolymer in ht-TGIC is different. An example for an ethene-octen block copolymer is shown. This block copolymer is subject of further investigations (2D Chromatography) to reveal all relevant information.

Because of the unique release mechanism ht-TGIC can be used to separate POLYOLEFIN blends when separation by HT-GPC fails.

Figure 3 shows the ethene segment distribution of an unknown blend.
As the overlay with reference substances shows, the sample is a blend of the 3 substances paraffin oil, LDPE, and a statistical copolymer. Paraffin oil is strictly separated according to GPC/SEC mode and elutes first. The main component, LDPE, has an average ethene sequence of about 2 500 g/mol. The third component can be identified as an statistical copolymer. It behaves like an ethene-octene copolymer with approximately 33% octene. However, when determining the CH_3/CH_{total} ratio, a higher proportion of CH_3 groups is found. Since the CH_3/CH_{total} ratio provides no structure information (length) for the comonomers, substance no. 3 may also be an ethene-butene (29% butene), an ethene-hexene (48% hexene), or an ethene-octene copolymer (67% octene).

Quantification of the individual components reveals the composition: 77.5% LDPE, 15.1% copolymer, and 7.4% paraffin oil.

Summary:
ht-TGIC combined with IR detection provides a powerful method for the separation of polyolefins based on their structure. The fact that it separates strictly according to the ethene sequence length makes it an ideal alternative for the separation of olefin blends, when HT-GPC cannot be applied. In addition to the separation structural parameters can be determined. This allows to assign the polymer type.

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PSS POLEFIN HT-GPC Columns

For HT-GPC separations of poly(ethylene), poly(propylene), and other polyolefins in TCB, o-DCB or Decalin.

Particle size:	Standard: 10 µm, others on request
Maximum Temperature:	200 °C
Maximum Pressure:	100-150 bar depending on porosity
Recommended Flowrate:	0.5-1 mL/min
Maximum Flowrate:	2 mL/min
Applicable with light scattering detectors:	Yes

Available are analytical columns (8 mm ID x 300 mm length) with the following porosities:

Part number	Porosity [Å]	Molar mass separation range* [Da]
poa0830101e2	100	100 - 10 000
poa0830101e3	1 000	100 - 60 000
poa0830101e5	100 000	1 000 - 1 000 000
poa0830101e6	1 000 000	1 000 - 4 000 000
poa0830101e7	10 000 000	5 000 - 30 000 000
poa0830101lim	linear M	100 - 1 000 000
poa083010lxl	linear XL	1 000 – 4 000 000
poa080510	Precolumn	

* (Poly(styrene) based)

Recommended column combinations:
low molar masses: 1 000 + 1 000 + 1 000 Å
medium molar masses: 1 000 + 100 000 + 1 000 000 Å
high molar masses: 1 000 + 100 000 + 10 000 000 Å

Test also our HighSpeed Columns!

PSS Events 2012*

Face-to-face training

GPC/SEC Training
September 20.- 21. 2012

Hands-on Visco/LS
June 27.- 28. 2012

Software Training

WinGPC Report Designer
September 10. 2012

WinGPC Basic Training
September 11. 2012

WinGPC Viscometry/Light Scattering
September 12. 2012

WinGPC ChromPilot
September 13. 2012

WinGPC Compliance Pack
September 14. 2012

Single day booking available.

User meetings

Column user meeting
October 16. 2012

WinGPC user meeting
October 17. 2012 - free of charge

EcoSEC user meeting
October 18. 2012 - free of charge

All seminars in Mainz, Germany

Web-based Training

Webinars GPC/SEC Basic Training

May 09. - 10. 2012, 11:00 AM EDT

October 24. - 25. 2012, 11:00 AM EDT

Webinars WinGPC Refresher

Basic WinGPC Refresher:

June 15. 2012, 11:00 AM EDT

October 10. 2012, 11:00 AM EDT

Advanced WinGPC Refresher:

June 16. 2012, 11:00 AM EDT

October 11. 2012, 11:00 AM EDT

The Viscometry, Light Scattering, Triple detection WinGPC Refresher:

June 17. 2012, 11:00 AM EDT

October 11. 2012, 11:00 AM EDT

PSS Webinars

2D Polymer Analysis:

April 25. 2012

It's magic. The PSS Column Concept:

May 24. 2012

Conferences/Trade Shows

April 17. – 20. 2012
Analytica 2012 in München/Germany
Hall A1, Booth 223

June 18. – 22. 2012
ACHEMA 2012 in Frankfurt/Germany
Hall 4.1, Booth P48

June 27. – 28. 2012
Separation Science 2012
in Kuala Lumpur/Malaysia
Oral presentation by Adrian Williams

August 19. – 23. 2012
ACS Fall National Meeting & Exposition
in Philadelphia, PA/USA
Booth

December 02. – 03. 2012
Advanced Materials by Modular Strategies:
From Synthesis to Industrial Application,
Houffalize/Belgium
Oral presentation by Thorsten Hofe

*Official language: English

New PSS employee

Since September 2011 Dr. Michael Möller is working for PSS.
He is responsible for method development, method transfer, the installation of GPC/SEC systems and for supporting our customers worldwide.

Michael studied chemistry at the University of Münster and finished his PhD about the synthesis of peptide polymer conjugates in February 2011.

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PSS has representatives in the following countries:

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