TOSOH

TOSOH
THE CUSTOMER
MAGAZINE

NO.#01 2014

ANTIBODIES / APPLICATIONS / AWARDS

TOSOH BIOSCIENCE

02
EDITORIAL
DEAR READER

Dear reader, welcome to the summer 2014 issue of the Tosoh Bioscience customer magazine. The motto of this issue is Antibodies – Applications – Awards. It is featuring new products and applications of antibody manufacturing, such as our new high capacity Protein A resin and its performance when processing high titer feedstocks.

What about awards? First, our new TOYOPEARL AF-rProtein A HC-650F is involved in a process that has been submitted for 'Best Technology Application Downstream' at the 2014 BioProcess International Award, which will be awarded in October at the BPI International Conference, Boston. Second, one of the video clips featured in the last issue of this magazine - The Chromatography Y-Factor - was nominated for the Comprix Healthcare Communication Award. On May 23rd we went to Cologne with great hope and expectations. However, we did not win. But we will try again. We will soon present another video clip, again related to antibody manufacturing. One of the actors already sneaked into this magazine. Watch out and follow our YouTube Channel to be one of the first to see the new clip.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING I MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

FINALIST
COMPRIX 2014

CONTENT

IMPRESSUM

TOSOH BIOSCIENCE GMBH
IM LEUSCHNERPARK 4 I 64347 GRIESHEIM I T: +49 [0] 6155 7043700 I F: +49 [0] 6155 8357900 I INFO.TBG@TOSOH.COM

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03 WHAT'S NEW RESINS

TOYOPEARL HIGH-CAPACITY PROTEIN A RESIN AND MiniChrom COLUMNS

AFTER A SUCCESSFUL INTRODUCTION OF NEW SEC COLUMNS FOR mAb ANALYSIS LAST SUMMER WE ARE HAPPY TO ANNOUNCE SOME INSPIRING PRODUCT INTRODUCTIONS FOR ANTIBODY PURIFICATION AND BIOPURIFICATION IN GENERAL. THE FAMILY OF ALKALI STABLE TOYOPEARL PROTEIN A RESINS WAS EXPANDED BY AN ULTRA-HIGH CAPACITY RESIN. IN ADDITION, THE MOST POPULAR TOYOPEARL AND TSKgel RESINS ARE NOW AVAILABLE IN A NEW COLUMN FORMAT, THE 5 mL MiniChrom COLUMNS FOR PROCESS DEVELOPMENT, PARAMETER SCREENING AND MICRO SCALE PURIFICATIONS.

TOYOPEARL AF-rProtein A HC-650F is a new Protein A chromatography resin designed for the purification of monoclonal antibodies (mAbs). It is well-suited for high capacity capturing of immunoglobulin out of high titer feedstocks and achieves 30% to 50% greater antibody adsorption than similar products. It exhibits dynamic binding capacities of greater than 70 g/L at residence times of 5 minutes, and greater than 50 g/L at 2 minutes with feedstock titers from 1 g/L to more than 10 g/L.

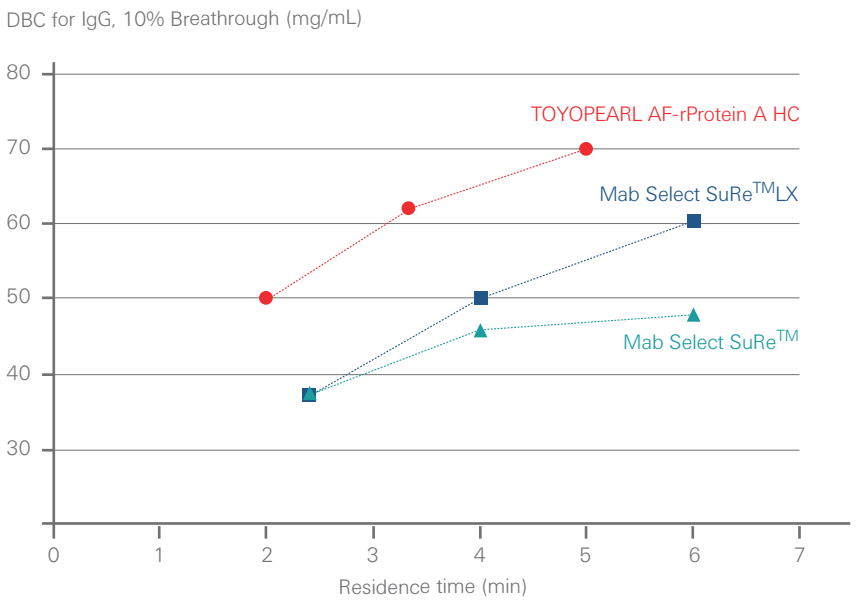
The recombinant ligand that is linked to the well-proven methacrylic polymer backbone of TOYOPEARL media has been engineered to maintain capacity even after repeated exposure to alkaline solution. Its multipoint attachment to the TOYOPEARL matrix further enhances chemical and thermal stability. In practice this pays off for a low level of Protein A leaching and also for a high resistance to alkaline solutions applied in cleaning-in-place (CIP) procedures.

Protein A resins constitute a substantial cost in state-of-the-art mAb purification processes. Factors such as operating cycles, capacity, and mAb titer have an impact on total costs associated with mAb purification. The high capacity of the new TOYOPEARL AF-rProtein A HC-650F and its high alkaline resistance increase product throughput, reduce operating costs, and increase manufacturing productivity.

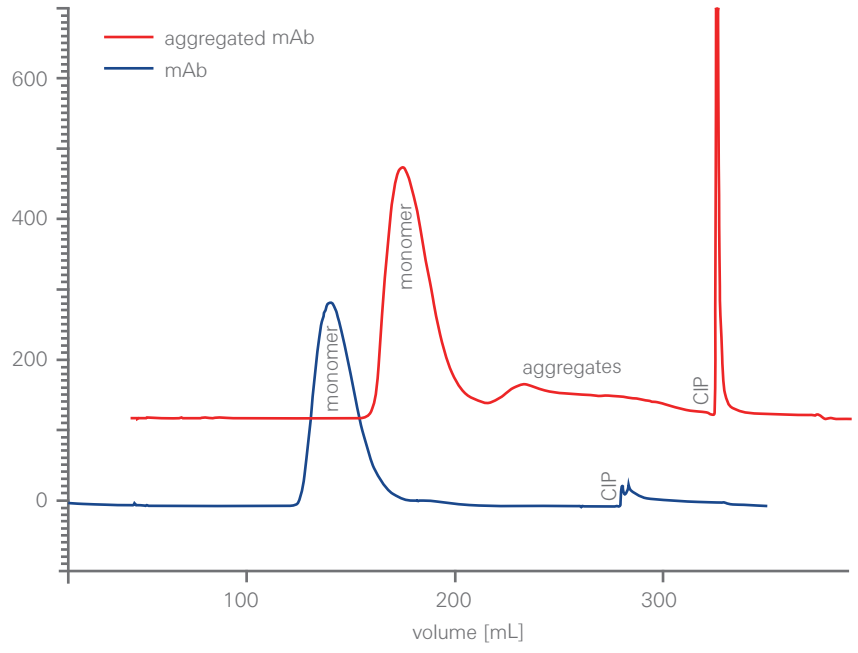
MiniChrom for TOYOPEARL and TSKgel are part of the method development platform from Tosoh Bioscience. They are designed for method optimization, parameter screening, and/or small scale purification. The columns (5 mL, 8 mm ID x 10 cm L) are made of biocompatible polyethylene and polypropylene and are prepacked with a range of TOYOPEARL or TSKgel chromatography resins under optimum compression, ensuring consistent experimental results. MiniChrom columns can be connected directly to any laboratory liquid chromatography system via standard HPLC connectors.

The 5 mL MiniChrom columns are the ideal tool to further optimize the purification method and to confirm the operational window after having selected a resin for a certain purification task by resin screening, e.g. with ToyoScreen RoboColumns on robotic workstations. The columns are packed by AtoIl GmbH and are available with a broad range of ion exchange, hydrophobic interaction, mixed-mode, gel filtration, and Protein A affinity media.

WIN A TOYOPEARL AF-rProtein A HC-650F 5 mL MiniChrom COLUMN AT HTTP://SVY.MK/1FLC3V9



COMPARISON OF BINDING CAPACITIES OF THREE ALKALI STABLE PROTEIN A RESINS

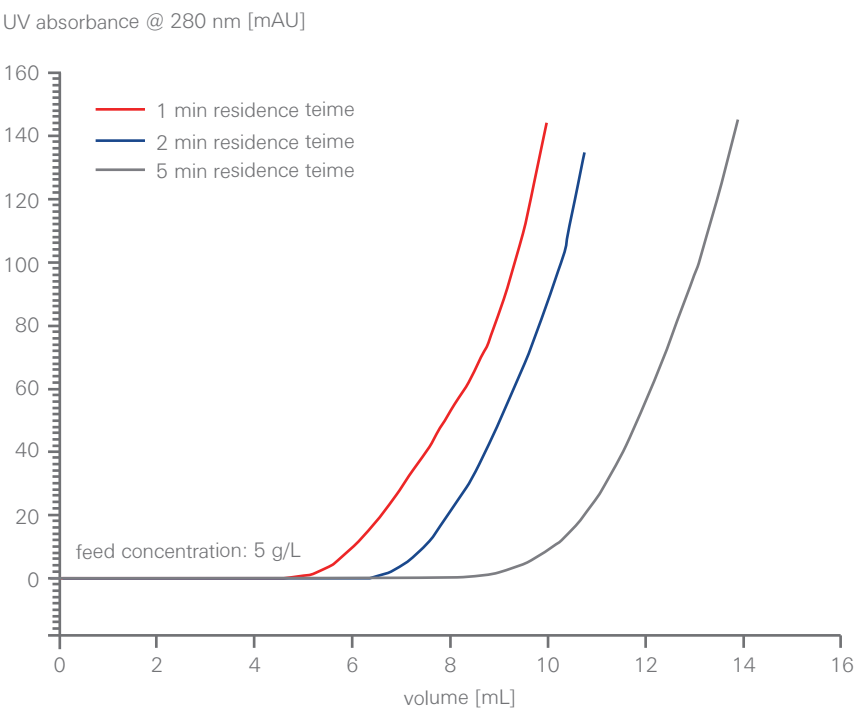


MIXED-MODE SEPARATION OF ANTIBODY AND AGGREGATES ON MiniChrom TOYOPEARL MX-Trp-650M

04
APPLICATION
ANTIBODY PURIFICATION

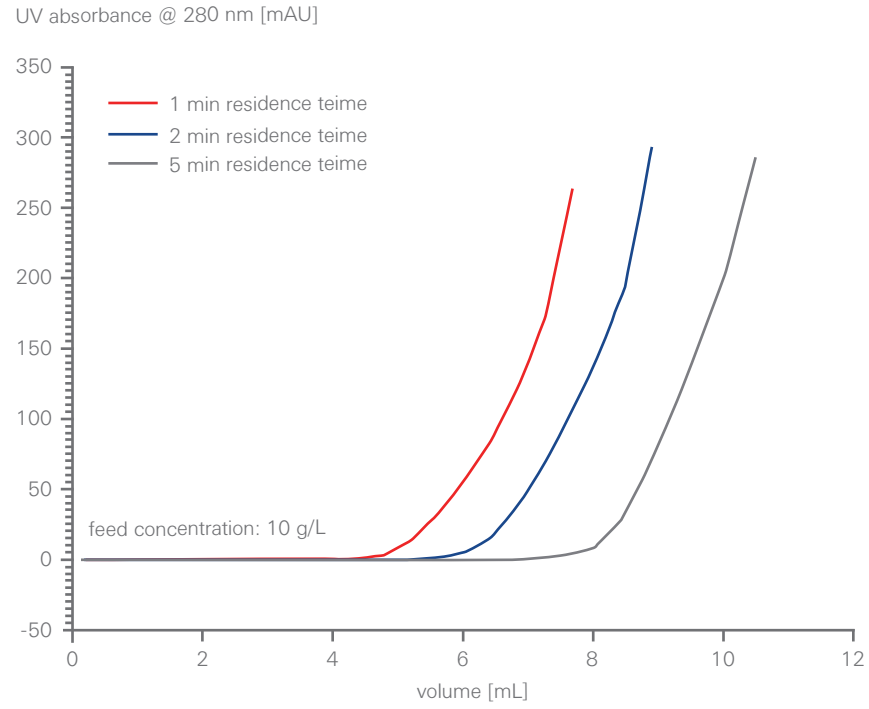
PROTEIN A CHROMATOGRAPHY WITH HIGH TITER FEEDSTOCKS

PROTEIN A CHROMATOGRAPHY HAS BECOME A WIDELY USED PLATFORM IN MONOCLONAL ANTIBODY (mAb) PURIFICATION. IT MAKES USE OF THE SPECIFIC INTERACTIONS THAT TAKE PLACE BETWEEN THE FC REGIONS OF IMMUNOGLOBULIN G AND IMMOBILIZED PROTEIN A, A CELL WALL COMPONENT OF STAPHYLOCOCCUS AUREUS. HEREIN, WE DESCRIBE THE IMPACT OF COMPARABLY HIGHER mAb LOADINGS ON AGGREGATION DURING PROTEIN A CHROMATOGRAPHY, AS WELL AS THE BENEFITS OF THE NEW ULTRA-HIGH CAPACITY TOYOPEARL AF-rProtein A HC-650F CHROMATOGRAPHY RESIN FOR HIGH TITER FEEDSTOCKS.



MAbs combine high target affinity and specificity with various different effector functions, such as complement activation. They are increasingly used in diagnostics and therapy. While the number of approved mAbs for pharmaceutical purposes has grown tremendously, mAb production processes have improved, too. Today, high mAb titers achieved through advanced expression technologies have become challenge in downstream processing, especially with regards to the expensive Protein A chromatography step.

In Protein A chromatography the antibody binds to the stationary phase while the remaining host cell proteins (HCP) of the expression cell line flow through the column and are usually reduced by two to three orders of magnitude. Subsequent mAb elution requires a pH shift of the mobile phase to pH 3-4. These non-physiological conditions are further applied for acidic virus inactivation but may cause mAb aggregation. The new TOYOPEARL AF-rProtein A HC-650F carries a recombinant ligand that is stable in alkali solutions applied in industrial cleaning procedures. It benefits from superior mAb capacity and increases capturing productivity. Besides higher capacities, the mAb uptake behavior is a major driver in process economics. High capacities at high feed concentrations will lead to concerting effects when it comes to fast and efficient capturing solutions.



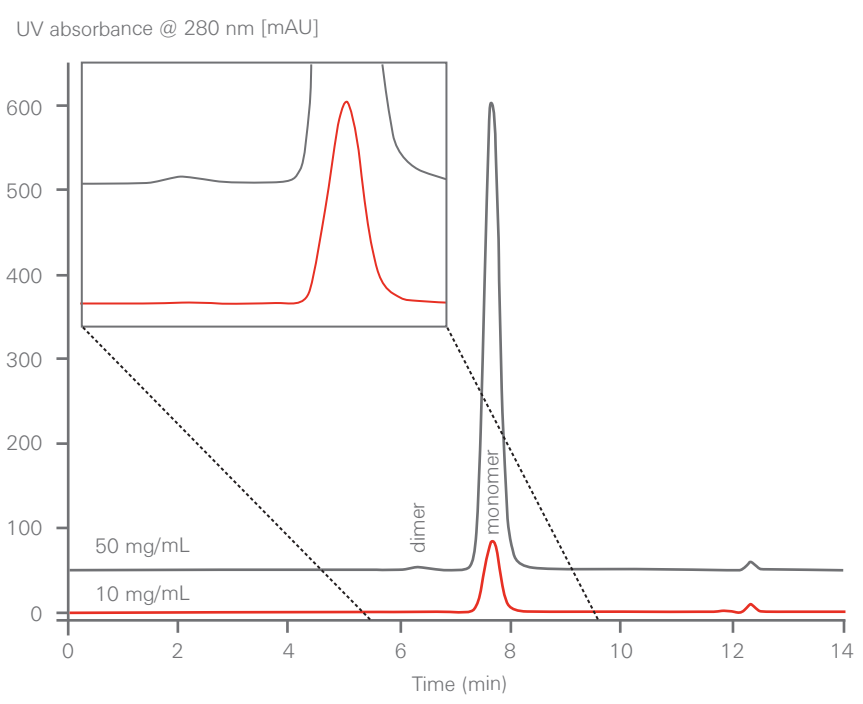


FIGURE 1A & B: DYNAMIC BINDING CAPACITY

Breakthrough curves of mAb A on TOYOPEARL AF-rProtein A HC-650F packed into a 6.6 mm ID X 2 cm L column; residence time 1 min, 2 min, 5 min A: 5 g/L mAb A; UV_{max}:1315 mAU. B: 10 g/L mAb A; UV_{max}: >2000 mAU.

FIGURE 2: AGGREGATE CONTENT

Size exclusion chromatogram of two Protein A elution pools. Feed: 4.75 g/L IgG to a total loading of 10 mg/ml and 50 mg/ml, Elution: 100 mM acetate buffer, pH 3.25, 50 L of the respective pools analyzed using TSKgel SuperSW mAb HR

The dynamic binding capacity (DBC) of a stationary phase is influenced by the contact time between the sample and the ligand, the so-called residence time. Good mass transfer properties enable a resin to reach a high binding capacity even at high flow rates. The capacity of the new resin was tested at various residence times and mAb titers. Figure 1a & b show the breakthrough curves for TOYOPEARL AF-rProtein A HC-650F at two feed concentrations. The resin shows complete mAb adsorption until breakthrough occurs. This remains unaffected even for short residence times of 1 min. The measured capacities of more than 100 mg/mL exceed the DBCs of all other base stable Protein A resins.

To further evaluate the elution properties, a purified humanized monoclonal IgG was diluted to a final concentration of 4.75 g/L. Simulating high HCP density in the feed, aliquots were spiked with concentrated cell culture fluid. Protein A chromatography with TOYOPEARL AF-rProteinA HC-650F was conducted 200 µL RoboColumns using a robotic chromatography station. The total loaded mass was varied from 10 to 50 mg/mL resin. A residence time of 2 minutes was applied. Before elution, the columns were washed with 20 column volumes of loading buffer.

MAb elution with acetate buffer, pH 3.25 delivered more than 95 % mAb recovery. Due to the acidic pH applied for elution, mAbs are prone to aggregation. Naturally, high capacity Protein A resins adsorb large amounts of mAb. This might enhance mAb aggregation due to higher protein concentrations in the elution pool. Thus, special attention was paid to the aggregate content after elution of the bound antibody.

Size exclusion chromatograms of two mAb elution pools are shown in Figure 2. The elution pools of 10 mg/mL and 50 mg/mL mAb load were injected, respectively. Although the SEC chromatograms seem to show aggregates for the higher loading only, a closer look reveals similar aggregate contents when referring to the corresponding total protein amount. Both pools contain 0.6 % aggregates.

In biopharmaceutical manufacturing, low Protein A ligand leaching is crucial and needs to be proved by ELISA testing. Protein A leaching was analyzed for 2.5 g/L and 7 g/L concentrated feed streams. Spiking and residence time were kept constant. Figure 3 shows that the absolute load has little influence on numeric Protein A leaching. Overall, Protein A leaching does not exceed 45 ppm for any of the tested pH and load conditions. Higher absolute mAb loadings seem to be advantageous, since the relative Protein A content of the mAb pool decreases.

Considering the obtained results regarding Protein A leaching, aggregate content and protein adsorption, high titers seem favorable for Protein A chromatography. This mAb seemed unaffected with regards to aggregation, and was efficiently adsorbed, which reduces Protein A cycle time. Further, Protein A leaching was even lower when applying higher titers. Thus, ultra-high capacity Protein A resins offer additional benefits besides reducing costs because less resin volume is needed to purify a given amount of monoclonal.

AUTHORS
JUDITH VAJDA, TOSOH BIOSCIENCE GmbH, ANGELIKA WACKER, UNIVERSITY OF APPLIED SCIENCES MANNHEIM

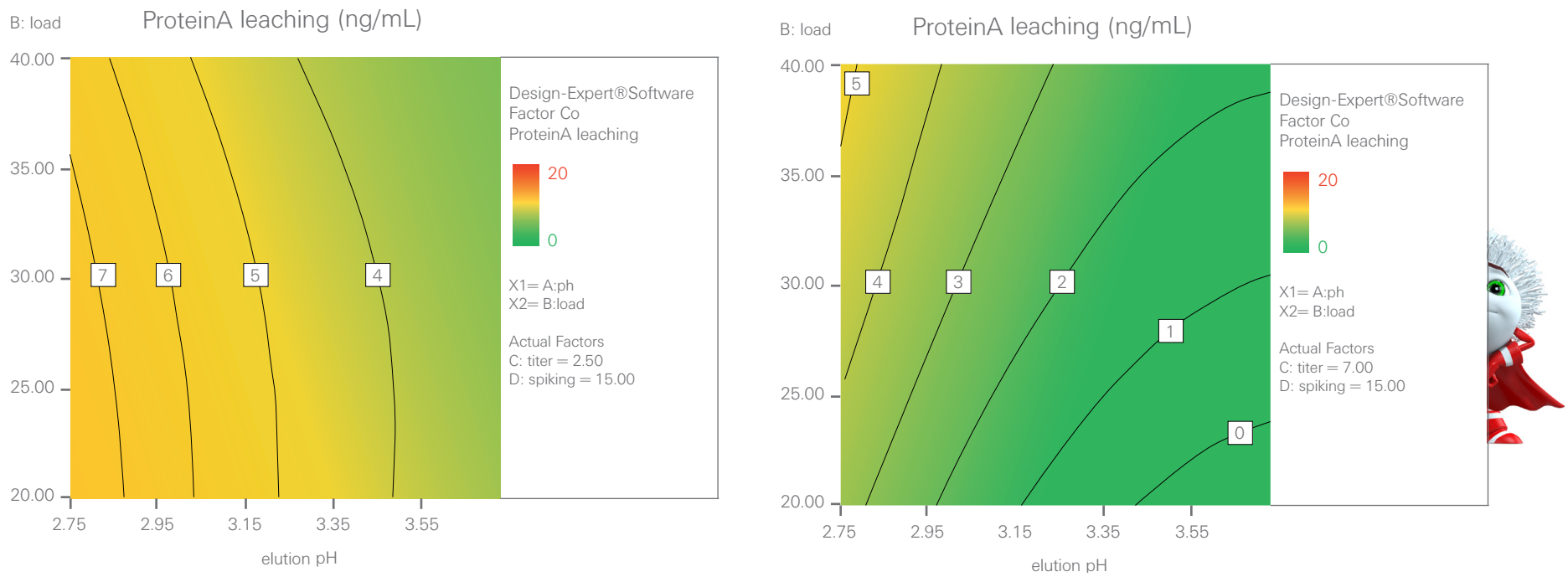
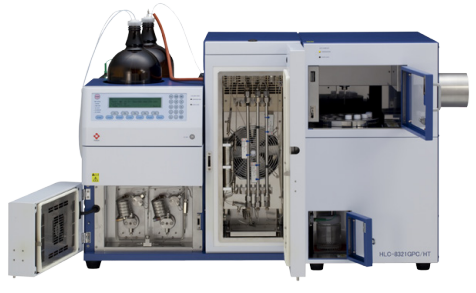


FIGURE 3: PROTEIN A LEACHING

Contour plots for TWO different load concentrations. Protein A leaching is plotted against pH and absolute load. A: 2.5 g/L. B: 7 g/L.

06 WHAT'S NEW GPC



HOT STUFF: HIGH TEMPERATURE GPC SYSTEM

A NEW COMPACT HIGH TEMPERATURE (HT) SYSTEM FOR GPC ANALYSIS AND CORRESPONDING GPC COLUMNS ARE THE LATEST ADDITION TO TOSOH'S POLYMER ANALYSIS PLATFORM. EcoSEC-HT, THE ALL-IN-ONE GPC/SEC SYSTEM FOR THE ANALYSIS OF ENGINEERING PLASTICS, PROVIDES STABLE THERMOSTATIZATION UP TO 220° C. THE TSKgel GMH$_{HR}$-HT2 GPC COLUMNS WERE DEVELOPED TO IDEALLY COMPLEMENT THE EcoSEC-HT.

Engineering plastics, such as ultra-high molecular weight polyolefins or polyphenylene sulfides, excel other materials by their mechanical strength and their resistance to chemical and physical degradation. These properties turn into hurdles when it comes to characterization of the polymers. They are crystalline and require elevated temperatures or special solvents for complete dissolution. The GPC analysis of engineering plastics at high temperatures requires specialized instruments and columns as high temperature is required throughout the whole experiment to avoid re-crystallization and to ensure that the sample remains in solution. The EcoSEC-HT GPC/SEC system provides stable thermostatization up to 220 °C. Integrated in a compact design it offers autoinjector, pumps, oven, and an extremely stable dual-flow refractive index (RI) detector with an independent temperature control.

The first step in high temperature polymer analysis is an efficient dissolution and filtration of samples prior to analysis. As the polymer chain is stressed by heat and shaking sample preparation needs to be very gentle. The best way to dissolve these polymers is moving the sample carefully and applying an individual temperature program until full dissolution has occurred. The optional DF-8321 sample processing unit can process up to 24 samples at temperature programs ranging from 40 to 220 °C. In addition samples can be filtered automatically to avoid clogging of the GPC column.

Refractive index (RI) detection is the standard detection for GPC/SEC applications. The new HT-GPC system offers a unique dual-flow RI detector. The combination of a high performance heating system and the dual flow RI detector speeds up equilibration time and reduces the baseline noise in a very effective way. Figure 1 shows the equilibration of column temperature and detector signal at a flow rate of 1 mL/min and a target temperature of 145 °C.

For optimum performance the GPC column should be carefully selected to ideally fit the temperature and solvent conditions. The TSKgel family offers a wide range of GPC/SEC columns with individual pore sizes but also mixed-bed and multi-pore columns exhibiting linear mass calibrations. The TSKgel GMH$_{HR}$-HT2 series was developed to complement the new high temperature GPC system when using the maximum temperature range up to 220 °C. Figure 2 shows a GPC analysis with RI-detection at 220°C.

The combination of HLC-8321GPC/HT and TSKgel GMH$_{HR}$ HT2 is a powerful tool for GPC measurement of engineering plastics. An accurate sample preparation is just as important as a stable RI detector baseline. A stable RI detector baseline is required for successful experiments in particular for repeatable and reproducible molar mass average calculations.

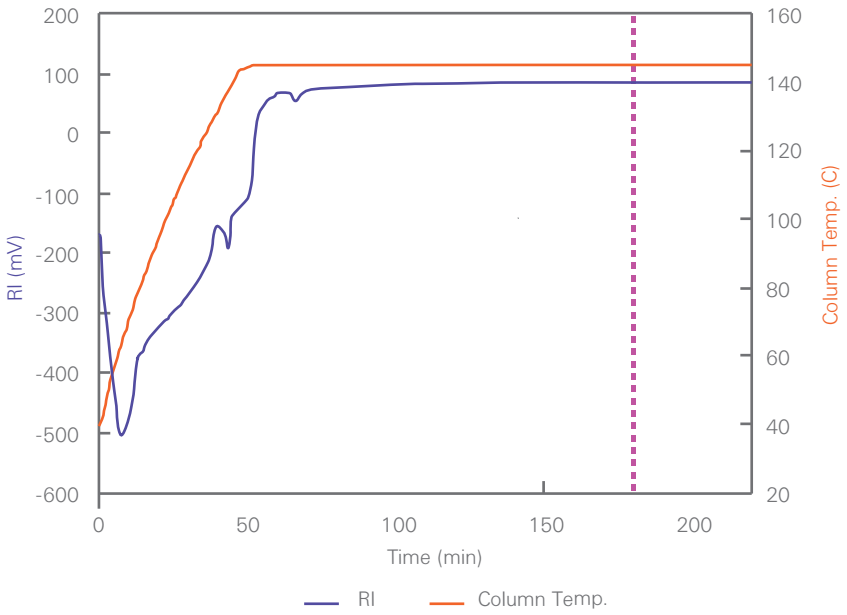


FIGURE 1: SYSTEM START-UP

Equilibration of temperature & RI signal

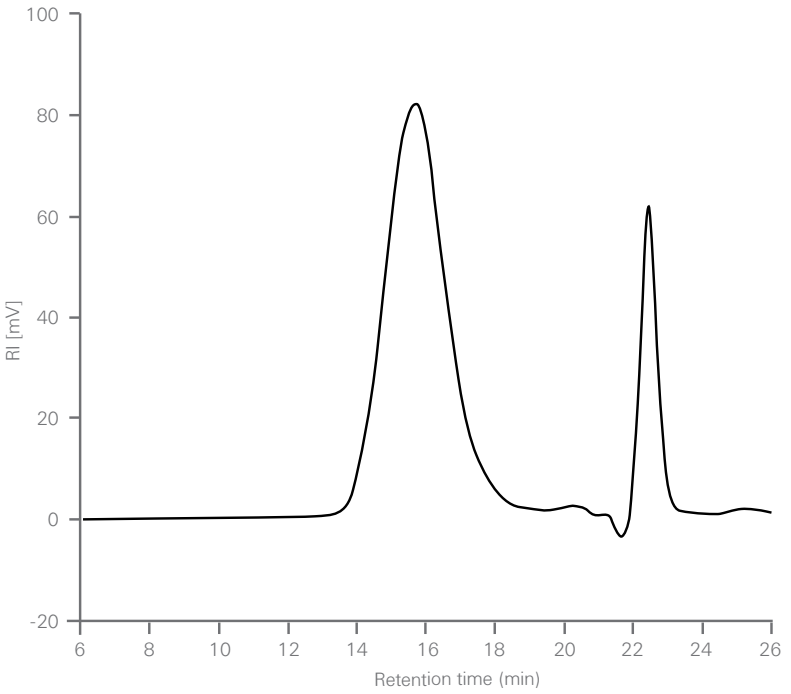


Figure 2: GPC ANALYSIS AT High Temperature

Polyphenylenesulfide (PPS) @ 220 °C, mobile phase: 1-chloronaphtalene; column: TSKgel GMH$_{HR}$-H(S)HT2 x 2

07 BIOSEPARATION FORUM

MEET THE EXPERTS AND DISCUSS CURRENT CHALLENGES IN BIOSEPARATION

TWO YEARS AGO WE STARTED A NEW SERIES OF ONE-DAY-SEMINARS ON BIOSEPARATION, THE BIOSEPARATION FORUM CHROMATOGRAPHY. AFTER SIX EVENTS IT'S TIME FOR A REVIEW.

The BioSeparation Forum series started in September 2012 at the Biotechnology Center IZB in Martinsried/Germany. The event provided the latest technical and scientific information about downstream processing to accelerate development and optimize production of biomolecules. With almost 80 participants from industry, academia and scientific institutes this event was a big success and we decided to expand this series over Europe. Today, we look back on six events, in Switzerland, the Netherlands, Austria, England and Germany. More than 250 guests attended these free of charge workshops and enjoyed a day full of biochromatography.

Typical topics covered were high throughput screening, process development and modern techniques in separation of monoclonal antibodies, recombinant proteins, plasma proteins and oligonucleotides. Additionally practical aspects in packing of columns have been presented as well as new chromatography technologies such as radial, continuous and counter current chromatography. The participants benefited from networking opportunities during coffee and lunch breaks to discuss specific technical challenges.

What distinguishe the Bioseparation Forum from other company seminars is that the broad mixture of topics is presented not only by specialists from Tosoh Bioscience but also by invited speakers from other suppliers, from academia and TOYOPEARL and TSKgel users from biotech industry and research. Suppliers of chromatography technology, such as Atoll GmbH, Proxcys BV, and service providers, e.g. ChromaCon AG and ViruSure, covered topics related to downstream processing. Renowned scientists, such as Marcel Ottens from the TU Delft or Jürgen Hubbuch from the Karlsruhe Institute of Technology (KIT), gave deeper insights to their field of research.

MORE PICTURES ON FACEBOOK:
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THE SERIES WILL BE CONTINUED WITH TWO EVENTS IN AUTUMN 2014:

- **OCTOBER 15 | 2014 — BERLIN [GERMANY]**
- **DECEMBER 02 | 2014 — DUBLIN [IRELAND]**

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WWW.SEPARATIONS.EU.TOSOHBIOSCIENCE.COM/WHATSNEW/CALENDAROFEVENTS/

08 CONFERENCE ANNOUNCEMENT

9TH HIC/RPC BIOSEPARATION CONFERENCE 2015

THE HIC/RPC CONFERENCE SERIES, WHICH ALTERNATES BETWEEN EUROPE AND THE USA, PROVIDES A UNIQUE FORUM FOR IN-DEPTH DISCUSSIONS ON DOWNSTREAM BIOPROCESSING. IT FOCUSES ON THE INCREASED SCIENTIFIC UNDERSTANDING OF THE HYDROPHOBIC NATURE OF BIOLOGICAL TARGETS AND THEIR CHROMATOGRAPHIC ISOLATION AND PURIFICATION. THE PROGRAM WILL PUT A SPECIAL FOCUS ON CURRENT DSP TOPICS SUCH AS ANTIBODY-DRUG-CONJUGATES OR MULTIMODAL CHROMATOGRAPHY.

The 9th HIC/RPC Bioseparation Conference will be held in Sliema, Malta, from March 16-19, 2015. Sliema is located close to Valetta, the capital of the Republic of Malta, the smallest member of the European Union. Malta International Airport (approximately 10 km from Sliema) has daily direct flights to and from the major European cities. On 316 square kilometres, Malta concentrates splendid witnesses to an eventful history that goes back to the Bronze Age and saw periods of Arabian, Italian, French, and British dominance.

Tosoh Bioscience is the sole sponsor of the conference and provides support for logistics and organisation for the scientific committee. The scientific committee of the 9th HIC/RPC conference will be headed by Professor Dr. Alois Jungbauer, University of Natural Resources and Applied Life Science (BOKU), Vienna, Austria.

VISIT THE HIC/RPC CONFERENCE WEBSITE – WWW.HIC-RPC.ORG

FOR ADDITIONAL CONFERENCE DETAILS, SUCH AS REGISTRATION INFORMATION, CALL FOR ABSTRACTS, AND OTHER INFORMATIONAL UPDATES.

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES

UPCOMING EVENTS

SEPT. 14 - 18	2014	ISC 2014	SALZBURG [AUSTRIA]
SEPT. 21 - 24	2014	5TH ICPC	VALENCIA [SPAIN]
SEPT. 30 - OCT. 02	2014	INTERNATIONAL SYMPOSIUM ON SEC/GPC	FRANKFURT [GERMANY]
OCT. 15	2014	BIOSEPARATION FORUM CHROMATOGRAPHY	BERLIN [GERMANY]
NOV. 05 - 07	2014	ISPPP 2014	WÜRZBURG [GERMANY]
DEC. 02	2014	BIOSEPARATION FORUM CHROMATOGRAPHY	DUBLIN [IRELAND]

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