TOSOH

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BIOTECHNICA / BIOMASS / BIOTHERAPEUTICS

TOSOH BIOSCIENCE

02
EDITORIAL
DEAR READER

Dear reader, welcome to the Biotechnica issue of the Tosoh Bioscience customer magazine. The motto of this issue is Biotechnica – Biomass – Biotherapeutics. It is featuring new applications and new products which will be presented at the Biotechnica tradeshow being held in Hanover from October 8 to 10. We are looking forward to meeting you there at our booth (Hall 009 B13).

Biotherapeutics are a key topic of our biannual 'Forum Prozesschromatographie', which will be held this November in Stuttgart. They are also the main targets when applying TOYOPEARL resins for various aspects of biopurification described in recent scientific publications (page 4). Another focus of this issue is GPC (gel permeation chromatography) applied in biomass research. We present some outstanding applications of our TSKgel GPC columns which were developed by the group of Dr. Roberto Rinaldi from the 'Max-Plank-Institut für Kohleforschung' in Mülheim, Germany.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING I MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

CONTENT

IMPRESSUM

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03 WHAT'S NEW RESINS

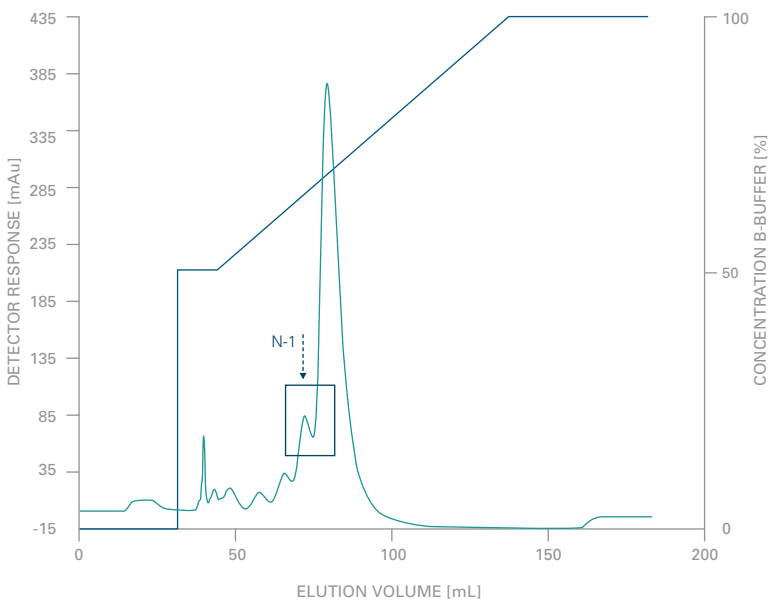
ADDITIONS TO THE TOYOPEARL GigaCap FAMILY

THE DEVELOPMENT OF HIGH EXPRESSION CELL LINES HAS TRIGGERED THE DEMAND FOR HIGH THROUGHPUT DOWNSTREAM PROCESSING. THIS REQUIRES RIGID RESINS THAT OFFER HIGH CAPACITIES AND EXCELLENT RECOVERIES AT HIGH FLOW RATES. THE RENOWNED TOYOPEARL GigaCap ION EXCHANGE RESINS MEET THESE NEEDS AND ARE IDEALLY SUITED FOR PURIFICATION OF MONOCLONAL ANTIBODIES AND OTHER BIOMOLECULES. RECENTLY TOYOPEARL GigaCap DEAE WAS INTRODUCED TO EXPAND THE CHOICE OF AVAILABLE FUNCTIONALITIES BY A WEAK ANION EXCHANGE RESIN. CUSTOMER DEMANDS FOR SMALLER PARTICLE SIZE VERSIONS OF THE EXISTING RESINS INITIATED THE DEVELOPMENT OF TOYOPEARL GigaCap S-650S AND Q-650S.

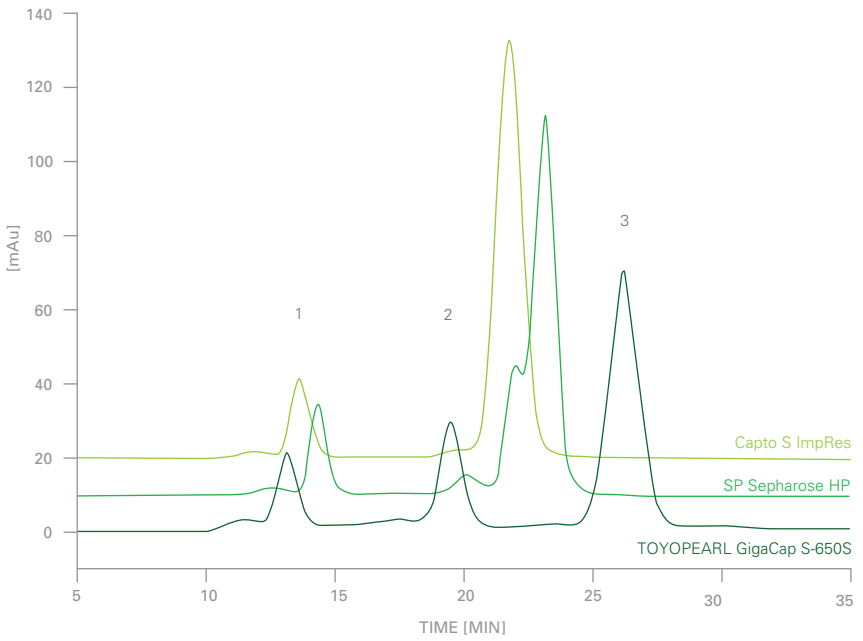
The TOYOPEARL GigaCap family consists of two cation exchange resins – GigaCap S-650 and GigaCap CM-650 – and the GigaCap Q-650 and DEAE-650 anion exchange resins. While all GigaCap media are available in M-grade particle size (75 µm) for capture and intermediate process steps, GigaCap S and Q are now also available in smaller particle size (S-grade; 35 µm) for high resolution intermediate and polishing purification.

➨ **TOYOPEARL GigaCap S-650S** is a strong cation exchange resin that combines advantageous pressure flow characteristics with excellent dynamic binding capacities (DBC) and high recoveries for a wide range of biomolecules. TOYOPEARL GigaCap S-650S offers a typical DBC of up to 165 g human IgG/L resin and a higher resolution than the 75 µm M-grade. It is ideally suited for high purity separations needed in final polishing steps. Good mass transfer kinetics enable the resin to maintain its dynamic binding capacity (DBC) at higher linear velocities. The figure below shows the high resolution achieved with TOYOPEARL GigaCap S-650S and its advantageous selectivity, when compared with agarose based cation exchange media with similar particle size.

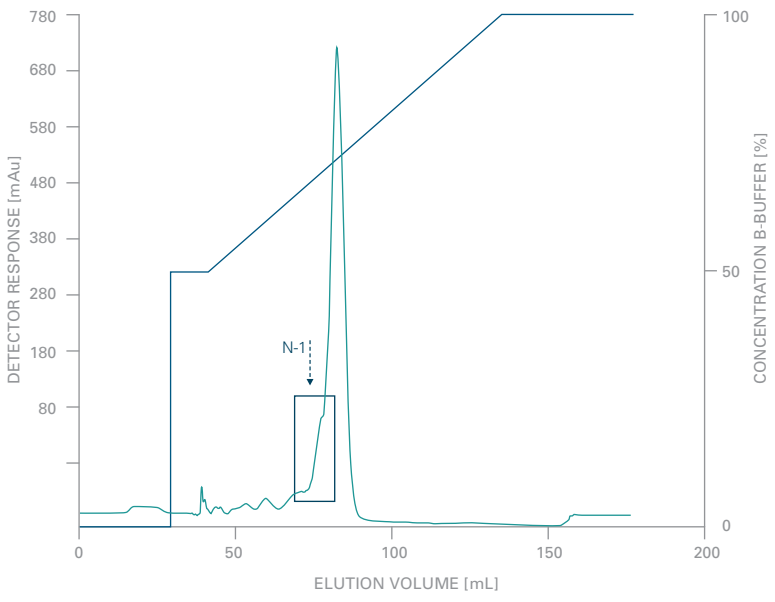
➨ **TOYOPEARL GigaCap Q-650S** is a strong anion exchange resin that offers DBCs approaching 190 g/L for BSA. This resin is ideal for separations that need a high resolution, such as the purification of oligonucleotides. The purification of oligonucleotides by anion exchange chromatography has traditionally fallen to resins such as TSKgel SuperQ-5PW (20) that offer high resolution and selectivity in conjunction with excellent mechanical stability at high column pressures. TOYOPEARL GigaCap Q-650S offers a low pressure alternative while preserving the selectivity, resolution and yields of those higher pressure processes. The figures below show that the N-1 peak was slightly better resolved with TSKgel SuperQ-5PW (20) than with TOYOPEARL GigaCap Q-650S. HPLC analysis of fractions taken across the peaks revealed that both resins were able to adequately resolve the full length oligonucleotide.



➨ PURIFICATION OF OLIGONUCLEOTIDES ON TSKgel SuperQ-5PW (20)



➨ SELECTIVITY OF SMALL PARTICLE SIZE CATION EXCHANGE RESINS



➨ PURIFICATION OF OLIGONUCLEOTIDES ON TOYOPEARL GigaCap Q-650S

04
TOYOPEARL
IN THE LITERATURE

PURIFICATION OF BISPECIFIC ANTIBODIES AND ANTIMICROBIAL ACTIVE PLASMA COMPONENTS

TOYOPEARL CHROMATOGRAPHY MEDIA ARE APPLIED IN A BROAD RANGE OF PURIFICATION SCHEMES OF BIOMOLECULES RANGING FROM NUCLEIC ACIDS TO PROTEINS. THE RESINS CAN BE APPLIED AT VARIOUS STAGES OF THE PURIFICATION PROCESS. EXAMPLES OF BIOTHERAPEUTICS PURIFIED AT LARGE SCALE WITH THE HELP OF TOYOPEARL ARE MONOCLONAL ANTIBODIES, EPOETIN (EPO), GROWTH FACTORS AND BLOOD PLASMA DERIVED PROTEINS, SUCH AS FACTOR VIII. THE USE OF TOYOPEARL IN RESEARCH AND METHOD DEVELOPMENT HAS BEEN DOCUMENTED IN MORE THAN 750 SCIENTIFIC PUBLICATIONS. WE CITE HERE SOME RECENT PUBLICATIONS WHERE TOYOPEARL RESINS WERE APPLIED FOR THE PURIFICATION OF BISPECIFIC ANTIBODIES AND OF ANTIMICROBIAL ACTIVE FRACTION OF CROCODILE PLASMA, RESPECTIVELY.

Thomas Müller-Späth and colleagues[1] developed a twin-column, countercurrent chromatography platform process for the purification of common light-chain (LC) bispecific antibodies. A bispecific antibody can bind two different antigens because its variable regions carry different specificities, which are generated by different heavy chains (HCs). Recombinant host cells for production of LC bispecific antibodies carry genes for both HCs, with the different specificities (A and B), along with one LC gene. A, B, and the light chains are expressed independently in those host cells, which then assemble them into three IgG types — AA, AB, and BB — for secretion into a culture environment. This mixture was captured from the feedstock by TOYOPEARL AF-rProtein A-650F which was chosen based on HCP clearance data. The purified IgGs were separated into the three different types by cation exchange multicolumn countercurrent solvent gradient purification (MCSGP), a proprietary chromatographic process that allows for product isolation with high yield and purity in difficult separation situations, and further polished by flow-through anion exchange chromatography on TOYOPEARL SuperQ-650M. Running the cation exchange step in MCSGP mode enabled isolation of the bispecific antibody AB at an 87% step yield. Overall yield of the chromatographic steps of the process was 78%. Thomas Müller-Spath will give a presentation on this exciting technology on the Forum Prozesschromatographie in Stuttgart (see page 8).

TOYOPEARL DEAE-650M was used by Jintana Kommanee and colleagues[2] to fractionate plasma from freshwater crocodiles in order to examine its antibacterial activity against pathogenic bacteria. Siamese crocodilians (Crocodylus siamensis) live with opportunistic bacterial infection but normally suffer no adverse effects. They are not totally immune to microbial infection, but their resistance thereto is remarkably effective. In this study, crude and purified plasma extracted from the Siamese crocodile were examined for antibacterial activity against clinically isolated, human pathogenic bacterial strains and the related reference strains. The crude plasma exhibited substantial antibacterial activities of more than 40% growth inhibition against the six reference strains of *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa,* and *Staphylococcus epidermidis,* and the four clinical isolates of *Staphylococcus epidermidis, Pseudomonas aeruginosa, Salmonella typhi,* and *Vibrio cholerae.* To further localize the antibacterial activity the plasma was fractionated by anion exchange chromatography on TOYOPEARL DEAE-650M into four fractions designated as fractions D1-D4. Only fraction D1 showed growth inhibition in the reference strains and the clinical, human pathogenic isolates. Subsequent studies are required to focus on the purification and characterization of the agent responsible for the antimicrobial activities including the anti-inflammatory activity.

REFERENCES

1 I MÜLLER-SPÄTH, TH. ET AL. PURIFYING COMMON LIGHT-CHAIN BISPECIFIC ANTIBODIES, BIOPROCESS INTERNATIONAL (2013), 11 (5), 36-45

2 I KOMMANEE, J. ET AL., ANTIBACTERIAL ACTIVITY OF PLASMA FROM CROCODILE (CROCODYLUS SIAMENSIS) AGAINST PATHOGENIC BACTERIA; ANNALS OF CLINICAL MICROBIOLOGY AND ANTIMICROBIALS (2012), 11:22 (HTTP://WWW.ANN-CLINMICROB.COM/CONTENT/11/1/22).

05 SEC APPLICATION

A TOOLBOX OF AMINO ACIDS FOR mAb SEPARATIONS

SIZE EXCLUSION CHROMATOGRAPHY (SEC) IS WELL ESTABLISHED FOR mAb AGGREGATE ANALYSIS. AS THE TECHNIQUE HAS BEEN USED SINCE THE EARLY DAYS OF MONOCLONAL ANTIBODY (mAb) DEVELOPMENT FOR PHARMACEUTICAL PURPOSES, VARIOUS METHOD IMPROVEMENTS HAVE EVOLVED. FOR INSTANCE, THE BENEFITS OF ARGININE ON ANALYTICAL SEC OF mAb AGGREGATE SAMPLES ARE WELL-KNOWN. A NEW APPLICATION NOTE SHOWS HOW SEC OF mAb AGGREGATE SAMPLES MAY TAKE ADVANTAGE OF OTHER AMINO ACID ADDITIVES IN THE MOBILE PHASE.

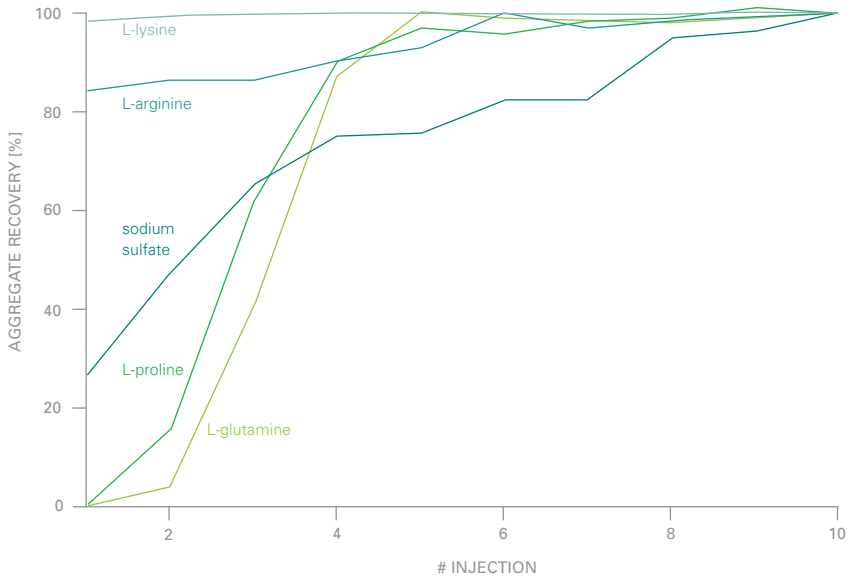
Recently, various approaches to improve analytical SEC have focused on reducing the analysis time. This can be achieved by staggered injection protocols or increased linear flow rates. The mobile phase composition itself leaves less room for method improvement, compared to other chromatographic modes. As soon as a certain ionic strength (inhibiting electrostatic interactions without causing hydrophobic interactions) and the pH of the mobile phase (ensuring structural integrity of proteins and the stationary phase) are set, one might think that the analysis solely depends on the particle size, packing quality and column length. However, the mobile phase composition is not at the end of its rope, in case the mentioned parameters have been set. For example, arginine and other amino acids can be added to the mobile phase to affect the aggregate recovery in SEC.

A mAb was aggregated by incubation at 75 °C for 5 min. Subsequently, the sample was analyzed with virgin TSKgel UltraSW Aggregate Columns (7.8 mm ID x 30 cm) with different mobile phases. 0.2 M lysine, arginine, proline, glutamine or sodium sulfate were added to 0.1 M sodium phosphate buffer, pH 6.7, respectively. A flow rate of 1 mL/min was applied, 20 µl (100 µg) of the aggregated mAb sample were injected onto equilibrated columns. The figure below illustrates the results on aggregate recovery. Glutamine and proline show a similar behavior: the aggregates are hardly recovered for the first two injections, while the aggregate peak suddenly appears for injection #3 and #4. The rise is not as sudden for sodium sulfate, but the aggregate peak will only achieve its full size for injection #10. In opposition to these results, lysine shows an even improved aggregate recovery compared to arginine. The inter-injection variability is low, depicting the complete aggregate content for all of the injections.

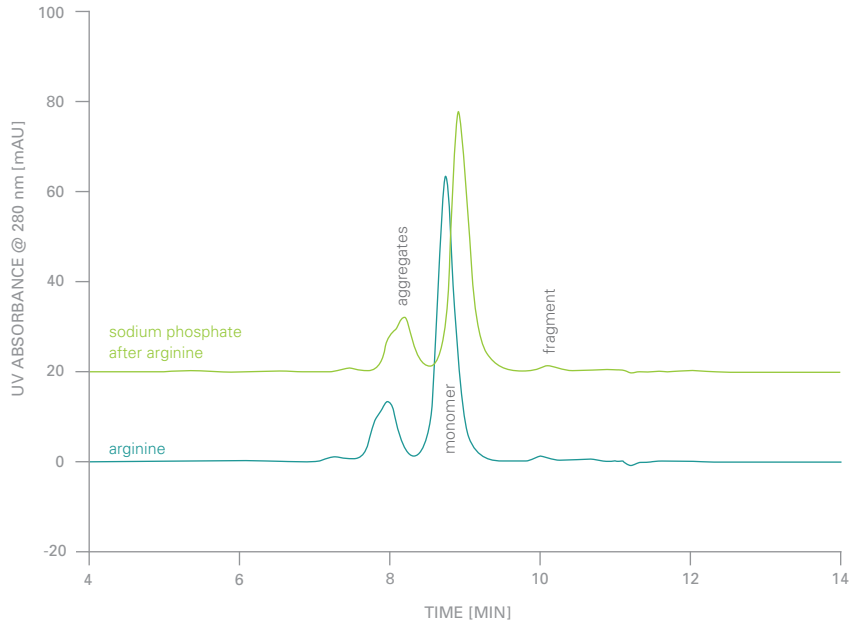
Besides aggregate recovery, resolution of the different sample components, namely the monomer and the different aggregates, is crucial for accurate analysis. Clearly, there is motivation to increase resolution. The figure below depicts the separation profile of an aggregated mAb sample on TSKgel UltraSW Aggregate using 0.1 M sodium phosphate buffer, pH 6.7 with an addition of 0.2 M arginine. 10 Injections with the arginine buffer were followed by 10 injections applying sodium phosphate buffer with an addition of 0.2 M sodium sulfate, in order to compare the two buffers. Monomer aggregate resolution, as well as monomer fragment resolution is slightly improved for the amino acid buffer.

Besides arginine, also proline and glutamine provide slightly increased monomer aggregate resolution. For arginine, the fragment monomer resolution is also improved. Although these increases in resolution are not drastic, they confirm that increased resolution due to the use of an advanced mobile phase is possible and that mobile phase testing can contribute to a more reliable and robust aggregate analysis.

CHECK OUT OUR WEBSITE TO DOWNLOAD THE FULL LENGTH APPLICATION NOTE.



AGGREGATE RECOVERY ON NEW TSKgel UltraSW AGGREGATE COLUMNS
The mobile phases contain different amino acids: lysine, arginine, prolin and glutamine. Sodium sulfate instead of an amino acid was added as a reference. Lysine and arginine allow almost complete aggregate recovery starting with injection #1, while proline and glutamine lead to reduced aggregate recovery compared to sodium sulfate.



SEPARATION PROFILE OF AN AGGREGATED MAB SAMPLE
Mobile phase: 0.1 M sodium phosphate buffer containing 0.2 M arginine. After 10 injections, the mobile phase was switched to sodium phosphate buffer with an addition of 0.2 M sodium sulfate. For both mobile phases, injection #10 is shown.

06
SEC/GPC
NEWS FROM THE FIELD

SEC/GPC ANALYSIS IN BIOMASS RESEARCH

THE 'MAX-PLANCK-INSTITUT FÜR KOHLENFORSCHUNG' IN MÜLHEIM, GERMANY – FAMOUS FOR THE WORK ON POLYETHYLENE SYNTHESIS BY KARL ZIEGLER RESULTING IN SEVERAL PATENTS AND THE NOBLE PRIZE FOR CHEMISTRY IN 1963 – PURSUES BASIC RESEARCH IN ALL FIELDS OF CATALYSIS. THE CENTRAL THEME PERVADING ALL DEPARTMENTS IS BASIC RESEARCH IN THE CATALYTIC TRANSFORMATION OF COMPOUNDS AND MATERIALS WITH THE HIGHEST DEGREE OF CHEMO-, REGIO- AND STEREOSELECTIVITY UNDER CONDITIONS WHICH MAXIMIZE EFFICIENT USE OF NATURAL RESOURCES. DR. ROBERTO RINALDI AND COLLEAGUES (GROUP HETEROGENEOUS CATALYSIS, PROF. DR. FERDI SCHÜTH) ARE WORKING ON BIOMASS CONVERSION. WE HAD THE CHANCE TO MEET DR. RINALDI IN HIS LAB TO TALK ABOUT THE APPLICATION OF TSKgel SEC/GPC COLUMNS IN THIS FASCINATING RESEARCH AREA.

Due to the expected depletion of fossil fuels, alternatives are required for the supply of our societies with fuels and alternative feedstocks for chemical production. Different types of biomass are suitable for this, but lignocellulose is the preferred option, since there is no competition with food and feed. The work in the group of Dr. Rinaldi is focussed on the depolymerization of cellulose and further conversion of the resulting sugars. The depolymerization of cellulose, our largest reserve of green carbon, is the critical step in the production of biofuels and chemicals from lignocellulose. Dr. Rinaldi and colleagues showed that the impregnation of cellulosic substrates with catalytic amounts of strong acid is a highly effective strategy to fully convert cellulose in a solvent free process into water-soluble oligosaccharides[1].

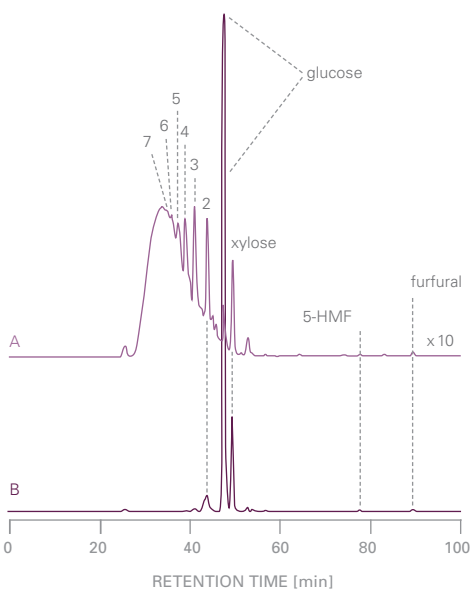
Mass spectrometry and size exclusion chromatography were used to characterize the soluble oligosaccharides and monitor their conversion into glucose and xylose in a subsequent acid hydrolysis step. ESI-MS spectra showed that the depolymerization process was effective for the conversion of α-cellulose to a complex mixture of water-soluble oligosaccharides containing glucose, xylose, and levoglucosan units, mostly comprising 3-6 sugar units. The oligosaccharides were further hydrolyzed at 130°C, resulting in 91% conversion of glucans into glucose and 96% of xylans into xylose. The figure below shows the water soluble oligomers obtained by depolymerization of cellulose (A) and the sample after 1 hour of acid hydrolysis (B). The numbers (n) indicate the degree of polymerization of oligosaccharides (Glcn). The separation was performed at 80°C with a set of four TSKgel G-Oligo-PW columns (6 µm, 7.8 mm ID x 30 cm) in series.

Besides TSKgel PW series columns used for the analysis of watersoluble oligomers, Dr. Rinaldi also successfully applied TSKgel SuperHZ series semi micro GPC columns for samples dissolved in organics, e.g. when analyzing the solvent effects on the hydrogenolysis of lignin with Raney Nickel[2]. The figure below depicts the GPC analysis (2x TSKgel Super HZ1000, 1x SuperHZ2000, 1x TSKgel SuperHZ3000 in series at 60°C with THF as mobile phase, photodiode array detection) of thermally treated lignin in a) methanol, b) 2-propanol, c) methylcyclohexane (MCH), and of d) organosolv lignin. The UV-absorption spots at around 275 nm indicate the presence of phenolic species in the solution, produced by heating a suspension of lignin in various solvents up to 300 °C. The higher retention time of the products obtained in MCH shows that smaller phenolic fragments are formed in the aprotic nonpolar solvent MCH than in 2-propanol or methanol. The results demonstrate that the solvent plays a key role in directing the selectivity of the thermolysis of lignin.

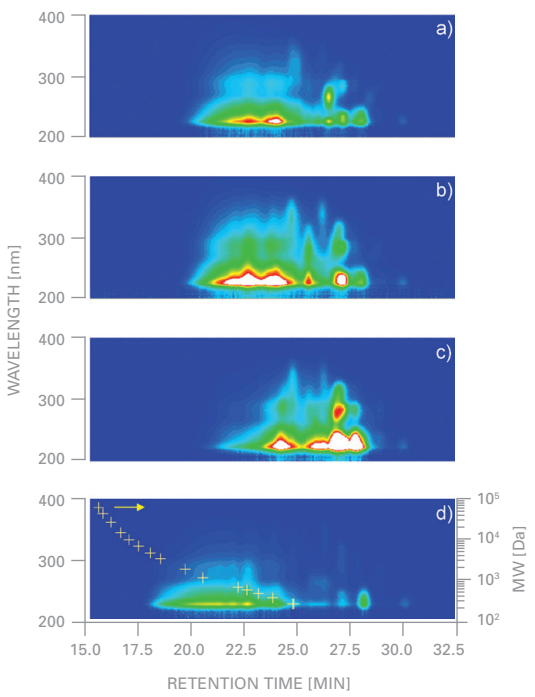
We are glad to see that our TSKgel SEC columns are applied in such a broad range of applications which may further contribute to the expanded use of cellulose based renewable resources in the production of biofuels and platform chemicals.

REFERENCES

1 I N. MEINE, R. RINALDI, & F. SCHÜTH, CHEMSUSCHEM 2012, 5, 1449-1454
2 I X WANG & R. RINALDI , CHEMSUSCHEM 2012, 5, 1455-1466



ACID HYDROLYSIS OF OLIGOSACCHARIDES



GPC ANALYSIS OF THERMALLY TREATED LIGNIN

07 CONNECT VIDEO CLIPS

AN EASY ACCESS TO CHROMATOGRAPHIC THEORY

WHEN A COMPLEX PROCESS IS NOT ONLY EXPLAINED AS GREY THEORY BUT GRAPHICALLY VISUALIZED IT IS MUCH EASIER TO UNDERSTAND AND REMEMBER IT. EVEN BETTER THAN SINGLE PICTURES, VIDEO CLIPS CAN DEPICT COMPREHENSIVE PROCEDURES. ON YOUTUBE YOU FIND VARIOUS TUTORIAL VIDEOS TAKEN IN THE CHROMATOGRAPHY LAB, SHOWING HOW TO USE OUR HPLC COLUMNS OR HOW TO PACK A PREPARATIVE COLUMN. BUT THESE VIDEOS CAN ONLY SHOW WHAT YOU SEE WITH YOUR EYES. WHAT ABOUT THE PROCESSES HAPPENING INSIDE A COLUMN? WELL, BESIDES OUR RENOWNED ANIMATIONS ABOUT ALL MODES OF CHROMATOGRAPHY THAT ARE USED ALSO IN OUR BASIC TRAINING COURSES, OUR NEW VIDEO CLIPS ON YOUTUBE CLOSE THIS GAP.

The new clips present some basics about chromatography in a neat and informative way. The two clips available so far illustrate preparative protein A affinity chromatography and analytical size exclusion chromatography. They are especially suited for users starting to use liquid chromatography for the purification or analysis of biomolecules. For experienced chromatographers the clips might at least remind them that chromatography is fun!

Our first video clip „What a Wonderful Chromatography Technique...“ illustrated how attractive monoclonal antibodies are especially for protein A ligands on chromatographic resins and how this appeal is exploited for the purification of monoclonals. The second clip „The Chromatography Y-Factor by Tosoh“ was just released and shows how the purified antibody has to prove its quality for example in a QC lab applying size exclusion chromatography to detect the content of aggregated antibodies and fragments.

CHECK OUT THE VIDEOS AND OUR ANIMATIONS ON THE MODES OF CHROMATOGRAPHY **ON YOUTUBE**.

08 CHROMATOGRAPHIC WORKSHOPS

FORUM PROZESSCHROMATOGRAPHIE – THE MICRO SYMPOSIUM ON DOWNSTREAM PROCESSING

IN ADDITION TO OUR RENOWNED BASIC WORKSHOPS ON CHROMATOGRAPHY IN PROCESS DEVELOPMENT AND PRODUCTION IN GERMAN AND ENGLISH LANGUAGE TAKING PLACE IN SEPTEMBER WE WILL ALSO HOST THE SO CALLED FORUM PROZESS CHROMATOGRAPHIE IN GERMAN LANGUAGE IN NOVEMBER. THIS MICRO SYMPOSIUM IS HELD EVERY SECOND YEAR AND PROVIDES PRAXIS RELATED PRESENTATIONS GIVEN BY EXPERTS FROM RESEARCH AND INDUSTRY. THE PROGRAM IS NEWLY ASSEMBLED FOR EACH EVENT IN ORDER TO TAKE UP CURRENT TOPICS IN DOWNSTREAM PROCESSING.

The Forum will take place on November 12 & 13 2013 in Stuttgart. This year's program covers various modes of chromatography applied for the separation and purification of biomolecules. A special focus will be multi modal chromatography with talks on 'Understanding Mixed Mode Chromatography' and on mixed mode separations of monoclonal immunoglobulin G and related molecules. Presentations on technical developments such as continuous chromatography and automated high throughput screening will alternate with application related talks ranging from plasmid analysis over purification of malaria vaccines to antibody purification and protein refolding. The Forum Prozesschromatographie is open for registration on our website www.tosohbioscience.de

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES OR JOIN ONE OF OUR RENOWNED WORKSHOPS

UPCOMING EVENTS

SEPT. 24 - 27 | 2013	ILMAC | BASEL [SWITZERLAND]
OCT. 08 - 10 | 2013	BIOTECHNICA | HANNOVER [GERMANY]
NOV. 19 | 2013	BIOSEPARATION FORUM - CHROMATOGRAPHY | STEVENAGE [UK]
NOV. 25 - 26 | 2013	NOVIA TAGE 2013 | BAD SODEN [GERMANY]
DEC. 05 | 2013	BIOSEPARATION FORUM - CHROMATOGRAPHY | VIENNA [AUSTRIA]
FEB. 11 - 12 | 2014	BIOINNOVATION LEADERS SUMMIT BILS 2014 | LONDON [UK]
APR. 01 - 04 | 2014	ANALYTICA 2014 | MUNICH [GERMANY]
APR. 02 - 03 | 2014	BIOPROCESS INTERNATIONAL EUROPE | PRAGUE [CZECH REPUBLIC]

TRAININGS | WORKSHOPS

NOV. 12 - 13 | 2013	FORUM PROZESSCHROMATOGRAPHIE ADVANCED COURSE IN GERMAN LANGUAGE | STUTTGART [GERMANY]
FEB. | 2014	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION | INTERNATIONAL COURSE IN ENGLISH LANGUAGE | STUTTGART [GERMANY]
MARCH | 2014	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION | BASIC COURSES IN GERMAN LANGUAGE | STUTTGART [GERMANY]