TOSOH

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SPRING / SCIENCE / SEPARATION

TOSOH BIOSCIENCE

02
EDITORIAL
DEAR READER

welcome to the first issue of the Tosoh Bioscience customer magazine in 2017. The motto of this issue is Spring/Science/Separation. It is featuring our conference event, the HIC/RPC Bioseparation Conference, which was held this spring in sunny Arizona, USA. About 100 participants enjoyed lively scientific discussions in a beautiful surrounding. The Biotechnica tradeshow will be relaunched under the name "LABVOLUTION", still taking place in Hannover, Germany, but shifted from autumn to spring (May 16-18). We will present our chromatography solutions at booth D35, hall 20. We are also looking forward to meeting you in June at the INCOREP, ISPAC or HPLC 2017 Conferences and enjoying more separation science.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING I MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

THE SUPER-T - COMIC #5

IMPRESSUM

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03 WHAT'S NEW RESINS

TOYOPEARL PROTEIN L RESIN FOR THE PURIFICATION OF ANTIBODY FRAGMENTS

A HIGH CAPACITY PROTEIN L RESIN COMPLEMENTS THE RANGE OF TOYOPEARL MEDIA FOR ANTIBODY PURIFICATION AND MANUFACTURING. THE NEW PROTEIN L AFFINITY RESIN, TOYOPEARL® AF-R PROTEIN L-650F, COMBINES THE RIGID TOYOPEARL POLYMER MATRIX WITH AN ENGINEERED RECOMBINANT PROTEIN L DERIVED LIGAND. IT ENABLES AN EASY PURIFICATION OF ANTIBODY RELATED TARGETS THAT CANNOT BE PURIFIED BY PROTEIN A CHROMATOGRAPHY.

Protein L based affinity chromatography is used for the capture of antibodies and antibody fragments that do not bind to Protein A. Unlike Protein A and G, which bind to the Fc region of immunoglobulins (IgGs), Protein L binds through interaction with the variable region of an antibody's kappa light chain. Therefore Protein L binds a wider range of antibody classes than Protein A. Figure 1 shows typical targets, such as antigen binding fragments (Fabs), single-chain variable fragments (scFvs) and domain antibodies (dAbs). Protein L also helps to purify those immunoglobulins whose Fc region is not easily accessible for Protein A, such as IgM or IgA.

TOYOPEARL AF-rProtein L-650F is an affinity chromatography resin that combines a rigid polymer matrix with a recombinant ligand, which is expressed in E.coli. Code optimization results in higher binding capacity and an improved stability of the ligand compared to the native Protein L molecule. The new resin provides the highest binding capacity available on the market for Fab molecules.

Figure 2 shows the binding capacity of TOYOPEARL AF-rProtein L-650F for a Fab fragment at various residence times in comparison to the most popular commercially available agarose based Protein L medium. Due to the excellent mass transfer characteristics of the new resin, especially dynamic binding capacities at 1 to 3 minutes residence time excel those obtained with the agarose-based resin. As affinity resin costs represent a considerable part of the overall production costs the high binding capacity of the new protein L resin can help to improve process economics in the production of antibody related recombinant molecules.

"TOYOPEARL AF-RPROTEINL-650F IS AN INNOVATIVE AND VERY USEFUL CHROMATOGRAPHIC RESIN IN MY PURIFICATION TOOLBOX, AS IT ALLOWS CAPTURE OF MULTIPLE ANTIBODY TYPES. IT IS THE RESIN I'VE BEEN EXPECTING FOR MANY YEARS." DR. MICHAEL DAVIDS - CEO DAVIDS BIOTECHNOLOGIE GMBH

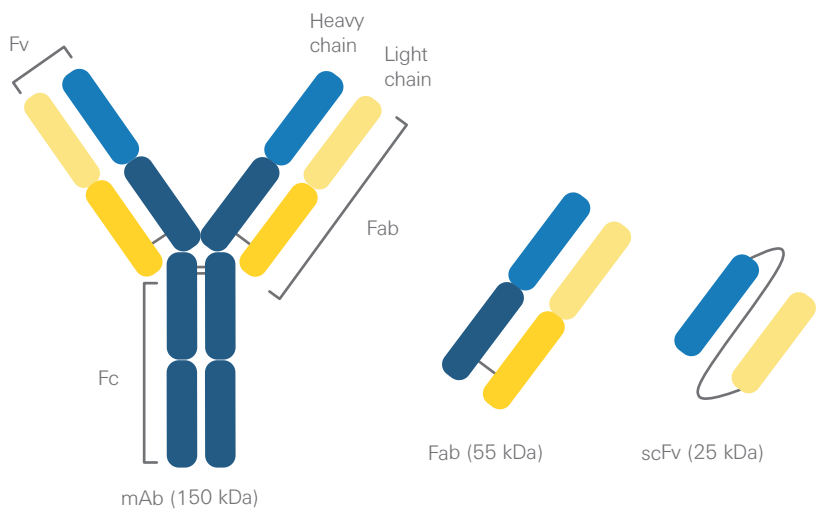

FIGURE 1: **TARGETS FOR PROTEIN L AFFINITY CAPTURING**

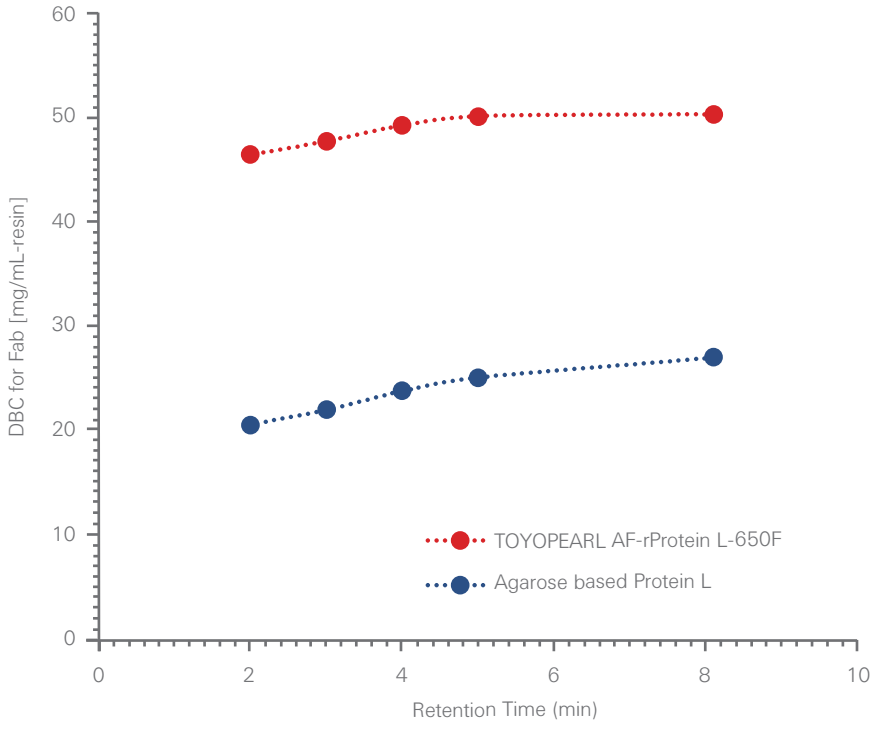

FIGURE 2: **BINDING CAPACITY OF PROTEIN L RESINS**

04 CONFERENCE REVIEW

10TH HIC/RPC HYDROPHOBIC BIOSEPARATION CONFERENCE IN SCOTTSDALE, ARIZONA

THE HIC/RPC CONFERENCE SERIES, WHICH ALTERNATES BETWEEN EUROPE AND THE USA, PROVIDES A UNIQUE FORUM FOR IN-DEPTH DISCUSSION OF DOWNSTREAM PROCESSING. THIS YEAR THE TAGLINE OF THE CONFERENCE WAS "ADVANCEMENTS, APPLICATIONS, AND THEORY IN HYDROPHOBIC BIOPROCESSING - NEW IDEAS CREATE IMPROVED SOLUTIONS FOR HYDROPHOBIC BIOPROCESSING". BESIDES ITS TRADITIONAL FOCUS ON APPLICATIONS AND FUNDAMENTAL RESEARCH ON HYDROPHOBICALLY INFLUENCED MODES OF CHROMATOGRAPHY, THE CONFERENCE ALSO PRESENTED NEWS ON NON-CHROMATOGRAPHIC HYDROPHOBIC PROCESSING METHODS SUCH AS PRECIPITATION OR CRYSTALLIZATION.

The conference venue was at 'The Scotts Resort and Spa', Scottsdale. Scottsdale is located close to Phoenix, the capital of the State of Arizona. Scottsdale's climate is arid. Winters are mild and summers are extremely hot. In February temperatures were very comfortable between 20 and 25 °C during the day. The beautiful venue and surrounding of the Conference combined with an excellent scientific program created the ideal atmosphere to encourage the exchange of ideas and thoughts among the attendees.

The Scientific committee of the conference, headed by Todd Przybycien (Carnegie Mellon University, Pittsburgh, Pennsylvania) composed a striking program, balanced between fundamentals and industrial applications. The scientific program started with a session on modelling and molecular behavior of hydrophobic interactions. It covered the use of molecular modelling and simulation tools to interpret the adsorption behavior of biological macromolecules on separation media or their aggregation behavior in solution.

The second session focused on adsorptive media design and applications. Our colleague Egbert Müller (Tosoh Bioscience, Griesheim, Germany) presented results of acid-base titrations of ion exchange resins and water sorption measurements of HIC Resins to characterize and evaluate their mechanisms of interaction. Day one ended with a 'Rapid Fire Poster Session' chaired and moderated in a very unique and entertaining way by Ron Bates (Bristol-Myers Squibb, Syracuse, New York). Every presenter got seven minutes to present their work with a few slides.

The first session of day two was dedicated to high throughput process development, chaired by Jürgen Hubbuch from KIT (Karlsruhe, Germany). Ana Azevedo (Institute for Bioengineering and Biosciences, Lisbon, Portugal) gave an excellent introduction to using microfluidic approaches for high-throughput screening of chromatographic conditions. Her co-worker Ines Pinto presented an example for the miniaturization of chromatography: multiplexed screening of different chromatography media and target molecules was used for the rapid optimization of antibody separations.

A highlight of the afternoon session on non-chromatographic processing and alternative media was the talk of Anja Trapp from Rentschler (Laupheim, Germany) on caprylic acid based impurity precipitations for downstream processes. The precipitation step was implemented in a two-column antibody purification process after Protein A capturing to precipitate host cell proteins and product aggregates. The presented results demonstrated that Caprylic acid precipitation can be an excellent alternative to a chromatographic polishing step.

The third day of the conference was a very entertaining experience for all participants. Every attendee was encouraged to contribute to the program. In the morning, we participated in two sessions of round table discussion. Up to 10 participants per table were requested to exchange their ideas and thoughts about current trends, challenges and the future of hydrophobic technologies and to summarize their discussion in one sentence.

Hydrophobic interaction chromatography (HIC) was the topic of the first session while alternative hydrophobically influenced processing technologies were discussed in the second session. Discussions were very lively and leading to interesting statements.

A new item on the agenda of the HIC/RPC conference was the announcement of the first HIC/RPC Conference Dr. Yoshio Kato Memorial Award. Dr. Kato's lifetime dedication to the bioseparation field at Tosoh Bioscience led to many commercial products employed in various analytical and downstream purification applications. The award was presented to Professor Milton Hearn, Monash University, Australia, as esteemed scientist and recipient of numerous awards and honors and long-standing fellow and old friend of the HIC/RPC conference.

In keeping with tradition, the conference offered not only a high class scientific program but also various opportunities to network with colleagues. The guided desert tour on Wednesday afternoon was the highlight of the social program of the Conference. We headed out deep into the Sonoran Desert where the participants could drive an authentic, military-grade TOMCAR ATV through a bumpy and challenging off road path.

Tosoh Bioscience is the sole sponsor of this conference series and provides support for logistics and organization for the Scientific Committee. The 11th HIC/RPC Bioseparation Conference will be organized by Tosoh Bioscience GmbH and will take place in Switzerland in late winter/early spring 2019.

THE CONFERENCE WEBSITE WWW.HIC-RPC.ORG WILL KEEP YOU UPDATED

06 WHAT'S NEW AWARDS

THE CHEMISTRY OF INNOVATION – NOT JUST A TAGLINE

THE COMPANY SLOGAN OF OUR PARENT COMPANY TOSOH CORPORATION, JAPAN, IS 'THE CHEMISTRY OF INNOVATION'. NUMEROUS AWARDS GRANTED TO TOSOH DURING THE LAST MONTHS PROVE THAT OUR COLLEAGUES IN RESEARCH AND DEVELOPMENT LIVE UP TO THIS MOTTO. TWO AWARDS ARE RELATED TO CHROMATOGRAPHY PRODUCTS LAUNCHED IN 2016; BUT ALSO OUR SISTER COMPANIES TOSOH QUARTZ INC. AND TOSOH SMD INC. RECEIVED PRESTIGEOUS AWARDS.

The Medicine Maker Innovation Awards showcase the most impressive launches over the year. TOYOPEARL Sulfate-650F, our new ion exchange chromatography resin, was judged to be one of the Top 15 innovations 2016. TOYOPEARL Sulfate-650F is a strong cation exchange resin that exhibits high salt tolerance, while allowing for high-protein binding capacities across a wide range of pH values and conductivities. The resin is especially suitable for the purification of therapeutic antibodies. Current purification processes frequently require diafiltration or dilution steps before loading the target onto the first ion exchange column. Protein purification with TOYOPEARL Sulfate-650F can help potentially shorten and simplify the whole process as physiological salt concentrations can be tolerated during protein binding. The judges said: "By permitting protein adsorption under high salt conditions, this new ion exchange resin may reduce the number of operations during bioprocessing."

In March, Tosoh Bioscience LLC received a Pittcon Today's Excellence Bronze Award for the TSKgel Protein A-5PW HPLC column. Pittcon Today's Excellence Awards were designed to recognize innovations at the 2017 Pittcon Exposition. A panel of judges – consisting of thought leaders from across academia, industry and trade media – viewed all types of scientific innovations and selected this year's winners based on submissions' ingenuity, creativity, implementation and outcomes, as well as the products' projected impact on the industry. Out of a diverse pool of more than 80 product submissions, the TSKgel Protein A-5PW column received a "Bronze Winner" accolade. The TSKgel Protein A-5PW column was introduced to customers working in monoclonal antibody development last August. "We are very excited that the judges from the Pittcon Today panel acknowledged the innovative capabilities of the TSKgel Protein A-5PW column and we are pleased to receive such an esteemed honor," said Cara Tomasek, Leader of the Product Management Group. "As the biopharma industry grows, more researchers will look for a product that can accomplish high throughput analysis since many harvest cell culture samples must be screened for IgG titer in early monoclonal antibody development. Because of its high flow rate tolerance and durability, along with its exceptional lifetime, the TSKgel Protein A-5PW column is the ideal solution for the analysis of monoclonal antibodies."

Congratulation also to our sister companies Tosoh Quartz, Inc., an industry leader in the production of quartz glass products for use by the semiconductor industry, and Tosoh SMD, Inc., a leading global provider of thin film deposition materials and of solutions to manufacturers in the semiconductor, display, solar, and large-area coating markets. They were among 26 companies recognized by Intel as worthy of 2016 Supplier Continuous Quality Improvement (SCQI) Awards.

We are looking forward to continuing these success stories as there are other exciting new products in the pipeline for 2018. In March, our global marketing team traveled to the Tosoh Nanyo Complex production facility in Japan to meet with our R&D team and discuss the next generation of our product lines.

07
APPLICATION
HIGH TEMPERATURE GPC

GPC ANALYSIS OF POLYPROPYLENE RANDOM COPOLYMERS

UNDERSTANDING A POLYMER IS THE KEY TO THE DEVELOPMENTS OF INNOVATIVE PRODUCTS. MOST OF THE POLYMER PROPERTIES ARE ASSOCIATED WITH THE MOLECULAR WEIGHT DISTRIBUTION AND AVERAGE MOLECULAR WEIGHTS. GPC IS THE ONLY CHARACTERIZATION TECHNIQUE USED TO DETERMINE BOTH THE MOLECULAR WEIGHT DISTRIBUTION AND THE AVERAGE MOLECULAR WEIGHTS OF POLYMERS. THEREFORE, CHARACTERIZATION OF POLYMERS USING GPC IS HIGHLY REQUIRED TO TAILOR THE POLYMER PROPERTIES ACCORDING TO THE APPLICATION. THE ARTICLE SHOWS HOW TOSOH CAN SUPPORT TO TAILOR THE PROPERTIES OF YOUR POLYMER.

The polypropylene market is one of the largest and most versatile polymer markets today with over 50 million tons produced annually and sold into a wide variety of household and industrial applications. The variety of products requires versatility in mechanical, thermal and chemical properties. For this reason, depending upon the application, three major categories of polypropylene exist: homopolymer, block copolymer, and random copolymer. While homopolymer is the general purpose grade of polypropylene, block copolymer exhibits enhanced impact resistance. Random copolymer is more malleable and crystal clear and is often used in medical applications and contact lens production.

The EcoSEC High Temperature GPC System encompassing a dual flow refractive index detector was used to perform GPC analysis of a polymer sample using a series of TSKgel high temperature GPC columns. The polypropylene random copolymer (300 µL Injection volume) was analyzed at 140 °C and a flow rate of 1 mL/min using two TSKgel GMH$_{HR}$-H(20)HT2 columns (20 µm 7.8 × 300 mm) and a trichlorobenzene (200 ppm butylated hydroxyl toluene (BHT) added mobile phase. The number-, weight- and z-average molar mass values (M_n, M_w, and M_z) and polydispersity index, PDI, were calculated for a polypropylene equivalent via EcoSEC software by applying a Mark-Houwink constant. The obtained values are given in the table below. The figure below shows an overlay of three runs.

The reproducibility and reliability of the method were shown through both the very low variation in sample retention and superb baseline stability observed when overlaying three consecutive injections of the sample. The enhanced thermal, flow rate and dual flow RI detector signal stability of the EcoSEC High Temperature GPC System in combination with the excellent resolving power of the TSKgel GMH$_{HR}$-H (20) HT2 high temperature GPC columns produce reliable and highly reproducible data for high temperature GPC analysis.

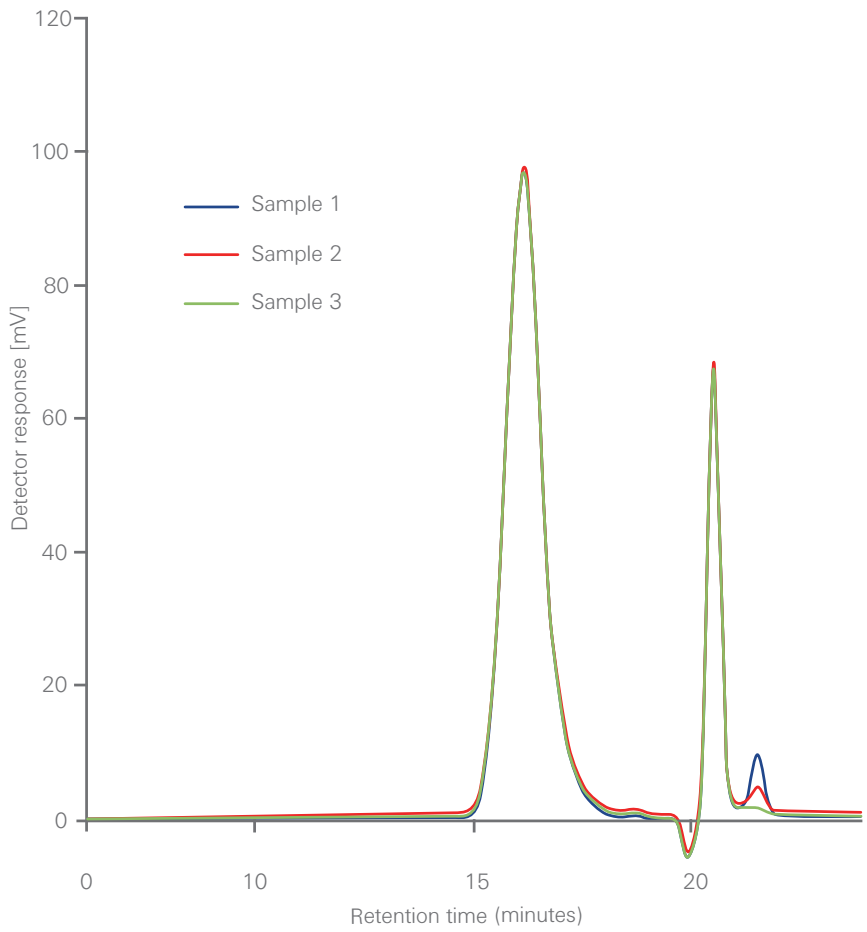

FIGURE: REFRACTIVE INDEX GPC ELUTION PROFILE OF 3 CONSECUTIVE INJECTIONS OF A POLYPROPYLENE RANDOM COPOLYMER SAMPLE (PP EQUIVALENT)

Injection number	Retention time (minutes)	Mn (g/mol)	Mw (g/mol)	Mz (g/mol)	PDI (Mw/Mm)
1	16.532	54380	145630	286074	2.678
2	16.527	54153	145548	289290	2.688
3	16.548	54027	145195	286331	2.687
Average	16.537	54187	145458	287232	2.684
STDEV	0.011	179	231	1787	0.001
CV%	0.066	0.33	0.16	0.62	0.200

TABLE: MOLAR MASS AVERAGES AND POLYDISPERSITY INDEX OF THE POLYPROPYLENE RANDOM COPOLYMER SAMPLE (PP EQUIVALENT)

08
WHAT'S HAPPENING
PRODUCTION CAPACITY

INCREASE OF PRODUCTION CAPACITY OF LIQUID CHROMATOGRAPHY SEPARATION AND PURIFICATION MEDIA

TOSOH CORPORATION HAS ANNOUNCED THAT IT WILL BE INCREASING THE PRODUCTION CAPACITY OF THE TOYOPEARL LIQUID CHROMATOGRAPHY SEPARATION AND PURIFICATION MEDIA AT THE NANYO COMPLEX, IN SHUNAN, YAMAGUCHI PREFECTURE. THE NEARLY 50% PRODUCTION CAPACITY INCREASE IS IN RESPONSE TO GROWING GLOBAL DEMAND FOR PURIFICATION MEDIA FROM THE ANTIBODY DRUG MANUFACTURING INDUSTRY.

At a cost of about 42 million, the Nanyo Complex's TOYOPEARL production facilities are being expanded. Construction began in October 2016 and is slated for completion in August 2018. Commercial operations at the expanded production facilities are expected to commence in April 2019.

The Nanyo Complex, Tosoh Corporation's main manufacturing hub, is located in the Yamaguchi Prefecture on Honchu Island in western Japan. This sprawling complex is nothing less than a self-contained city with its own power generation plants, port, and manufacturing facilities.

Given the rapid growth of the market for biopharmaceutical products, in Japan, the United States, in Europe, in China, in India, and in other emerging nations, chromatographic media are in high demand. Tosoh is committed to meeting that demand.

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES

UPCOMING EVENTS

MAY	16 - 18	2017	LABVOLUTION [BIOTECHNICA] 2017	HANNOVER [GERMANY]
JUNE	6 - 9	2017	INCOREP 2017	GELEEN/MAASTRICHT [THE NETHERLANDS]
JUNE	11 - 14	2017	ISPAC 2017	LINZ [AUSTRIA]
JUNE	18 - 22	2017	HPLC 2017	PRAGUE [CZECH REPUBLIC]

TRAININGS | WORKSHOPS

SEP.	19 - 21	2017	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION	BASIC COURSE IN GERMAN | STUTTGART | [GERMANY]
SEP.	26 - 28	2017	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION	BASIC COURSE IN GERMAN | STUTTGART | [GERMANY]
NOV.	22 - 23	2017	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION	BASIC COURSE IN ENGLISH | GRIESHEIM | [GERMANY]

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