TOSOH

TOSOH
THE CUSTOMER
MAGAZINE

NO. #02 2016

PERFORMANCE / PROCESS ANALYTICS / PROTEIN A

TOSOH BIOSCIENCE

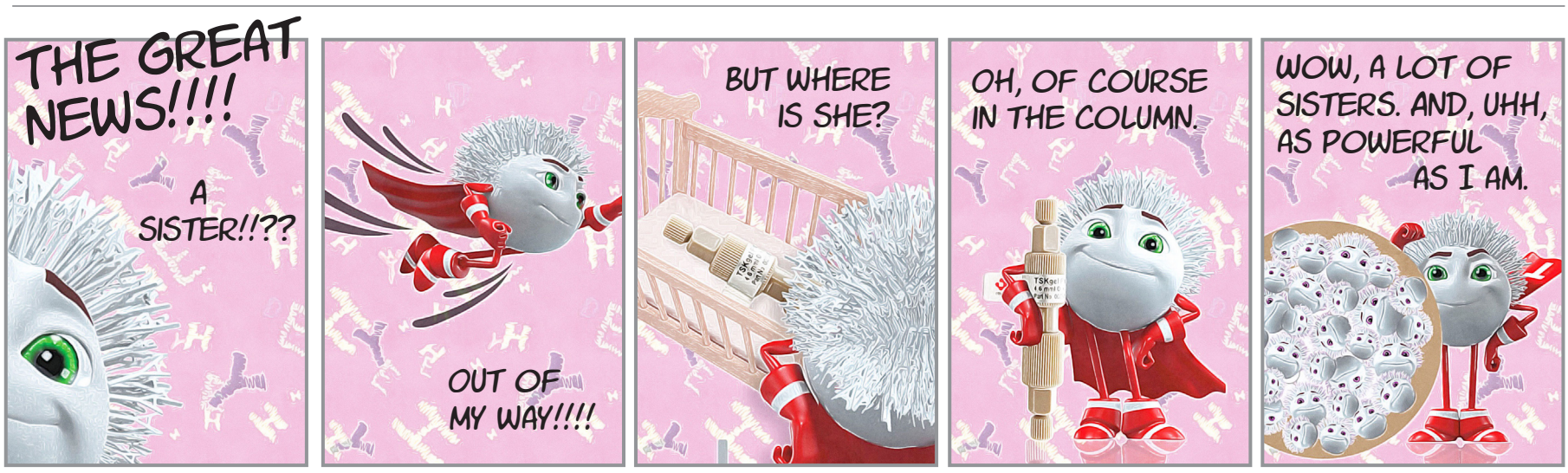
02 EDITORIAL DEAR READER

Dear reader, welcome to the autumn 2016 issue of the Tosoh Bioscience customer magazine. From September 20 to 23, ILMAC 2016 will take place in Basel, Switzerland. We invite you to visit our booth (Hall 1.1/A161) to discuss your separation needs with our specialists and check out the news from Tosoh. Besides our solutions for polymer analysis we will present a new HPLC column for IgG titer analysis and a new salt tolerant cation exchange resin.
The application presented in this issue of the customer magazine describes a process analytics method for the analysis of bispecific antibodies. At HPLC 2016 in San Francisco we discovered posters presenting interesting results obtained using latest column developments.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING I MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

THE SUPER-T - COMIC #4



GET TO KNOW TSKGEL PROTEIN A-5PW ON PAGE 3

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IMPRESSUM

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03 WHAT'S NEW TSKGEL

TSKgel PROTEIN A-5PW – FAST mAb TITER ANALYSIS

IN MANY STAGES OF MONOCLONAL ANTIBODY (mAb) DEVELOPMENT, HARVEST CELL CULTURE SAMPLES MUST BE SCREENED FOR IgG TITER. ANTIBODY TITER DETERMINATION BY PROTEIN A AFFINITY HPLC IS MUCH MORE ROBUST, RELIABLE AND REPRODUCIBLE THAN ENZYME-LINKED IMMUNOSORBENT ASSAYS (ELISAS). A NEW PROTEIN A COLUMN WAS SPECIFICALLY DESIGNED FOR FAST AND ACCURATE DETERMINATION OF MONOCLONAL ANTIBODY (mAb) CONCENTRATION DURING CELL LINE SELECTION OR UPSTREAM BIOPROCESS OPTIMIZATION AND CONTROL.

TSKgel Protein A-5PW expands the line of TSKgel columns for antibody analysis with a high performance affinity chromatography column. Protein A affinity columns can be employed to determine the concentration of a monoclonal antibody for the optimal time for harvesting or to identify clones that express the most antibodies. If necessary, even a partial purification for further analysis can be accomplished using a Protein A affinity column.

TSKgel Protein A-5PW is a 20 µm, 4.6 mm ID × 3.5 cm column for high performance affinity chromatography. Made of PEEK hardware, this column has been designed for rapid separation and robust quantification of a variety of antibodies. Monoclonal antibodies can be captured and accurately quantitated in less than 2 minutes per injection. As shown in Figure 1, IgG was separated well from impurities in CHO cell culture supernatant by stepwise pH gradient within 2 minutes. All host cell proteins from the supernatant are eluted in a flow-through peak and only IgG is captured and eluted by the column at approximately 1 minute retention time.

The wide range loading capacity of the TSKgel Protein A-5PW column can accurately determine the titer of mAb at various stages of mAb development: from initial screening in R&D to process control in upstream development. Its reproducibility of injection-after-injection allows the users to accurately monitor the titer of mAb with high confidence. In addition, the low level of Protein A leaching makes this column a good candidate for small scale purification of mAbs for initial characterization.

Figure 2 demonstrates the high durability and the wide dynamic range of the TSKgel Protein A-5PW column. The column was subjected to a linearity analysis test. Purified IgG was initially injected onto the column with subsequent injections of IgG made at different volumes. The column was then used up to 2,009 injections without being cleaned. A linearity analysis test was then repeated. No significant change in the calibration curve for IgG was seen. The column still maintained its high loading capacity with an excellent linearity (R^2=0.9999).

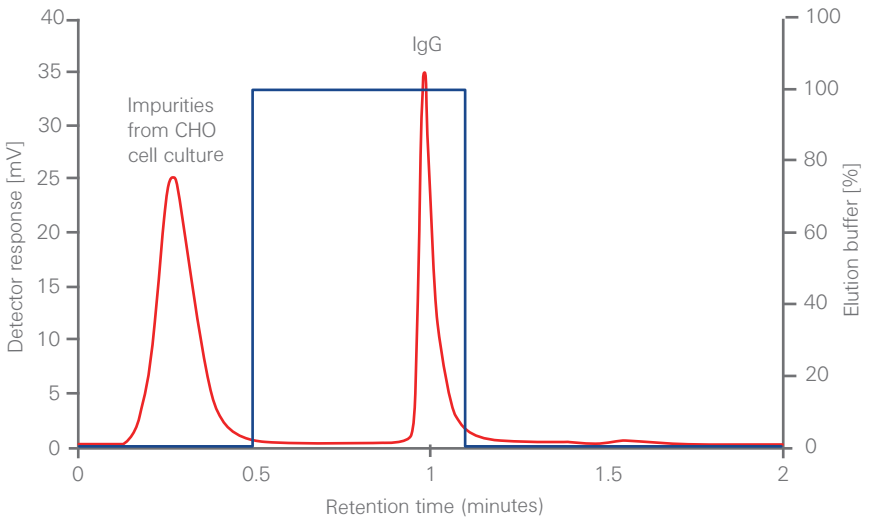

FIGURE 1: **RAPID SEPARATION OF IgG FROM IMPURITIES**

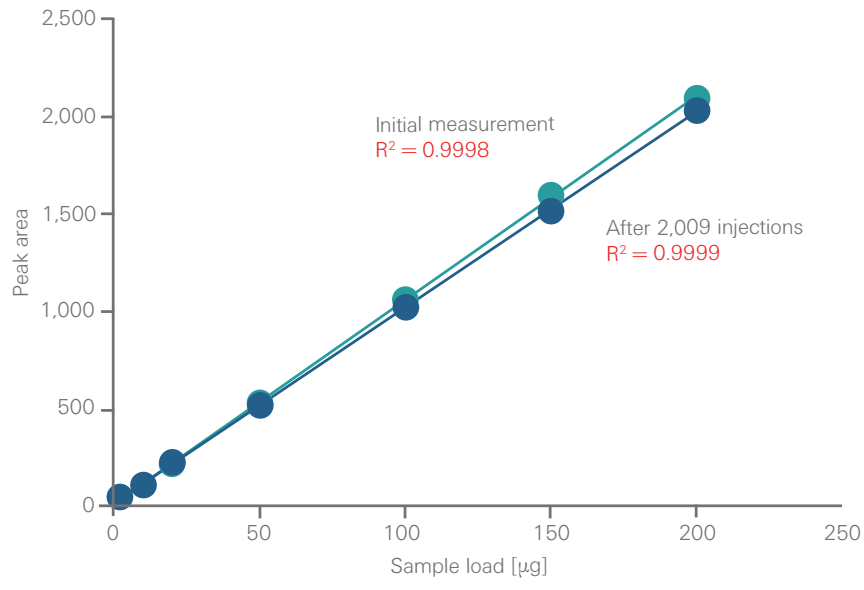

FIGURE 2: **DURABILITY AND DYNAMIC RANGE OF TSKgel PROTEIN A-5PW**

04
APPLICATION
BISPECIFIC ANTIBODIES

A PROCESS ANALYTICAL METHOD FOR BISPECIFIC ANTIBODIES

A FAST HIGH-RESOLUTION ANALYTICAL CATION EXCHANGE CHROMATOGRAPHY METHOD HAS BEEN DEVELOPED BY TOSOH BIOSCIENCE FOR IN-PROCESS QUANTIFICATION OF κλ-BODY, κκ-MONOSPECIFIC AND λλ-MONOSPECIFIC ANTIBODIES AT NOVIMMUNE.

Bispecific monoclonal antibodies combine different target specificities, and many different formats have already been developed. Novimmune, a Swiss biotech company, focused on the discovery and development of antibody-based drugs for the targeted treatment of inflammatory diseases, immune-related disorders and cancer, has developed the κλ-body concept using κ- and λ-light chains to support binding of different targets. κλ-bodies are unmodified fully human bispecific IgGs (Figure 1). In contrast to existing engineered formats, κλ-bodies are unique in offering the typical functional and biochemical characteristics of a human antibody[1]. Production of κλ-bodies in hybrid hybridoma cells leads to monospecific κκ and λλ by-products. A high resolution analytical chromatography method discriminating those three species is mandatory in order to assess the purity of the end-product.

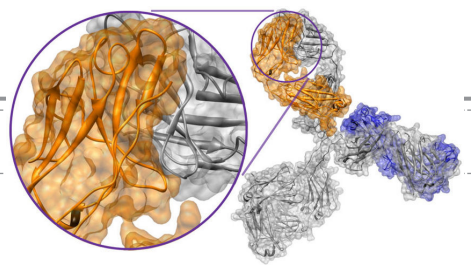

FIGURE 1:

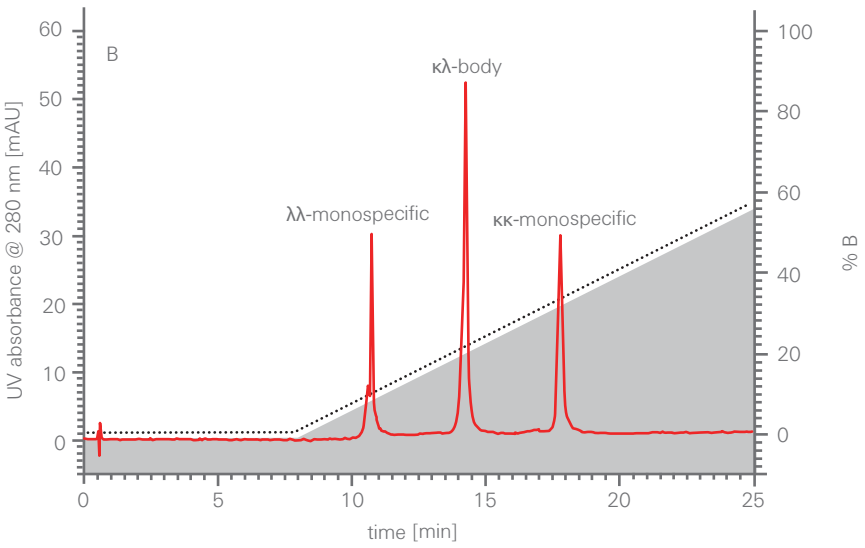

FIGURE 2B: **ION EXCHANGE CHROMATOGRAPHY**

A hydrophobic interaction chromatography based separation of the κκ-monospecific, λλ-monospecific, and the κλ-body on TSKgel Butyl-NPR is shown in Figure 2A. According to the affinity purified reference standard (data not shown), the first peak corresponds to the κκ-monospecific. The third peak represents the λλ-monospecific. The κλ-body elutes in between the two monospecific mAbs. Resolution of the κκ-monospecific and κλ-body is comparatively high. The λλ-monospecific elutes as a shoulder in the tail of the κλ-body. This order of elution further indicates that the λ-light chain is more hydrophobic than the κ-light chain. Hydrophobic interaction chromatography analysis can be accomplished in 30 min.

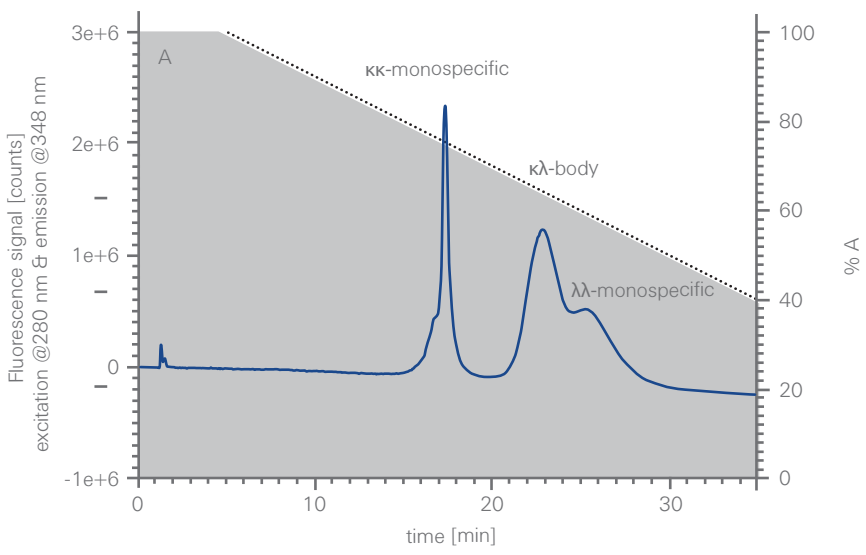

FIGURE 2A: **HYDROPHOBIC INTERACTION CHROMATOGRAPHY**

Comparatively higher resolution of the three mAb variants can be achieved with TSKgel SP-STAT (Figure 2B). The κκ-monospecific, λλ-monospecific, and the κλ-body are resolved to baseline in an 18 min run. A comparison with the reference standard indicates that the λλ-monospecific elutes first, followed by the κλ-body and the κκ-monospecific. The developed analytical cation exchange method allows separating the different monospecific variants and the κλ-body in roughly half the time required for analytical hydrophobic interaction chromatography.

This is most probably due to the faster mass transfer of cation exchange chromatography. The surface of the SP-STAT stationary phase is hydrophilized, which is known to be a reason for faster mass transfer[2]. Various commercialized preparative and analytical stationary phases take advantage of this effect. These media provide higher binding capacities and resolution. Due to these advantages over the previously developed hydrophobic interaction chromatography method, analytical cation exchange chromatography was used as the method of choice for process analysis.

[1] **HTTP://WWW.NOVIMMUNE.COM/PLATFORM/BISPECIFIC.HTML**

[2] **M. URMANN, H. GRAALFS, M. JOEHNCK, L.R. JACOB, C. FRECH, MABS 2 395–404.**

05
HPLC 2016 SE-UHPLC

UHPLC POSTERS PRESENTED AT HPLC 2016 IN SAN FRANCISCO

THIS YEAR, HPLC 2016, THE MAJOR ANNUAL EVENT FOR SCIENTISTS WHO USE AND DEVELOP HPLC, TOOK PLACE IN JUNE IN SAN FRANCISCO. BESIDES SEVERAL POSTER PRESENTATIONS BY TOSOH COLLEAGUES, WE FOUND VERY INTERESTING POSTERS FROM THE BIOPHARMA INDUSTRY EVALUATING RECENTLY INTRODUCED TSKgel COLUMNS. TSKgel UP-SW3000, THE 2 MICRON UHPLC COLUMN WITH 25 NM PORE SIZE FOR SIZE EXCLUSION CHROMATOGRAPHY WAS INTRODUCED IN 2015. IT IS THE LATEST ADDITION TO THE RENOWNED TSKgel SW SERIES OF SILICA BASED GEL FILTRATION COLUMNS, THAT ARE WIDELY USED IN RESEARCH AND INDUSTRY TO ANALYZE THE AGGREGATE CONTENT OF ANTIBODY BASED DRUGS.

Less than one year after the introduction of the silica based size exclusion chromatography (SEC) columns for aggregate analysis, several posters at the HPLC 2016 conference in San Francisco showed results generated with this exciting UHPLC column. The presenting authors came from notable biopharm manufacturers. The posters were part of the session: P-T-0100: Biopharma-Large Molecule and General Applications:

David Fulchiron et al., Analytical Operations at Genentech[1], evaluated the recovery of high molecular weight species (HMWS) on different SE UHPLC columns. He compared TSKgel UP-SW3000 to another SE-UHPLC column on the market and these both UHPLC columns to the gold standard in HPLC analysis of aggregates, the TSKgel G3000SWXL. Both UHPLC columns were shown to display superior resolution to the conventional HPLC column with reduced waste and reduced run time. For a non stressed sample, both UHPLC columns provided similar quantitation to the HPLC column. For a stressed sample, they observed non specific binding on the competitive column that was not observed in either of the TSKgel columns. When non-specific binding was observed, HMWS were under-recovered. The authors recommend verifying SE-UHPLC results by an orthogonal method to ensure recovery.

Xianwen Chen and colleagues from Boehringer Ingelheim Biopharma Fremont[2] presented the development of a high resolution size exclusion chromatography method for monoclonal antibody analysis. They used a TSKgel UP-SW3000 column from TOSOH with 2 µm particle size. Their existing SE-UHPLC method using another SE-UHPLC column with 1.7 µm particles size suffered from robustness issues, including column lot to lot variation and sometimes quick loss of resolution on low molecular weight components over limited usage. Several monoclonal antibodies were tested as case studies with both columns. The results obtained demonstrated that the new method using TSKgel UP-SW3000 showed comparable resolution with improved robustness and reduced cost.

Colleagues from Tosoh also presented posters in the same session: Chrystal Brenner and colleagues from Tosoh Bioscience LLC[3], in the US, showed data on the separation of free PEG from PEGylated mAbs and mAb fragments using the TSKgel UP-SW3000 UHPLC SEC column. Samples were analyzed both in conventional HPLC and UHPLC and a charged aerosol detector. Colleagues from our office in Griesheim presented the application data on bispecific antibodies[4] described on the previous page and the use of Amide-HILIC-UHPLC to verify an FcR affinity chromatography analytic of glycosylation patterns[5]. In a session on LC column technologies, colleagues from Tosoh Corporation in Japan presented data on the new TSKgel Protein A-5PW column that is described on page 3 of this magazine[6].

[1] HPLC 2016 P-T-0123: **SEPARATED BUT NOT EQUAL: A CASE STUDY OF VHMWS RECOVERY ON DIFFERENT SE-UHPLC COLUMNS; D. FULCHIRON, J. REA, B. WEI, J. JEONG; GENENTECH, SOUTH SAN FRANCISCO**

[2] HPLC 2016 P-T-0119: **DEVELOPMENT OF A HIGH RESOLUTION SIZE EXCLUSION CHROMATOGRAPHY FOR MONOCLONAL ANTIBODY ANALYSIS; XIANWEN CHEN, EIKE ZIMMERMANN, BOEHRINGER INGELHEIM, FREMONT, FREMONT, CA, USA**

[3] HPLC 2016 P-T-0119: **SEPARATION OF FREE PEG FROM PEGYLATED mAbs AND mAb FRAGMENTS USING A NEW SILICA BASED, 2 µM PARTICLE SIZE, 25 NM PORE SIZE UHPLC SEC COLUMN WHEN ANALYZED BOTH IN CONVENTIONAL HPLC AND UHPLC AND A CHARGED AEROSOL DETECTOR; CRYSTAL BENNER, ATIS CHAKRABARTI, TOSOH BIOSCIENCE LLC, KING OF PRUSSIA, PA, USA**

[4] HPLC 2016 P-T-0103: **ANALYTICAL SEPARATIONS OF BISPECIFIC MONOCLONAL ANTIBODIES WITH NONPOROUS RESINS. JUDITH VAJDA (1), JEAN-FRANCOIS DEPOISIER (2), ROMAIN DABRE (1), EGBERT MÜLLER(1), 1: TOSOH BIOSCIENCE GMBH, GRIESHEIM, GERMANY; 2: NOVIMMUNE SA, GENEVA, SWITZERLAND**

[5] HPLC 2016 P-T-1513: **USING AMIDE-HILIC-UHPLC TO VERIFY AN FCR AFFINITY CHROMATOGRAPHY ANALYTIC OF DIFFERENT mAbs ACCORDING TO THEIR GLYCOSYLATION PATTERNS. WERNER CONZE, EGBERT MUELLER, TOSOH BIOSCIENCE GMBH, GRIESHEIM, GERMANY**

[6] HPLC 2016 P-T-0222: **FAST QUANTIFICATION OF IMMUNOGLOBULIN G USING A NEW PROTEIN A ANALYTICAL HPLC COLUMN. HARUMI OKUMURA, SATOSHI FUJII, KOSUKE ARAKI, SHIGERU NAKATANI,TOSOH CORPORATION, YAMAGUCHI, JAPAN**

06
WHAT'S NEW
TOYOPEARL

TOYOPEARL SULFATE-650F – THE SMART CATION EXCHANGE RESIN

ION EXCHANGE CHROMATOGRAPHY (IEC) IS ONE OF THE MOST FREQUENTLY USED CHROMATOGRAPHIC MODES FOR THE SEPARATION AND PURIFICATION OF BIOMOLECULES. IT IS USED AT ALL STAGES AND SCALES OF PURIFICATION OF THERAPEUTIC PROTEINS: FROM LABORATORY SCALE PURIFICATION TO INDUSTRIAL SCALE DOWNSTREAM PROCESSING. THE NEW SALT TOLERANT TOYOPEARL SULFATE-650F REPRESENTS THE CATION EXCHANGE COUNTERPART TO OUR EXTREMELY SUCCESSFUL SALT TOLERANT ANION EXCHANGE RESIN TOYOPEARL NH2-750F AND COMES WITH EVEN MORE EXTRA VALUE IN TERMS OF SELECTIVITY.

Modern ion exchange resins offer extremely high binding capacities. The interest is now shifting towards salt-tolerant ion exchange media that enable capturing out of a biological feedstock at physiological conditions or direct processing of target fractions without dilution. Compared to other cation exchange resins, TOYOPEARL Sulfate-650F provides salt tolerance plus a unique selectivity. The resin is especially suited for mAb aggregate removal and in addition provides affinity like interaction to other targets.

TOYOPEARL Sulfate-650F is based on the proven TOYOPEARL matrix which is functionalized with sulfate groups. It offers a high binding capacity for IgG at a broad range of salt concentrations. Figure 1 shows the effect of buffer pH and sodium chloride concentration on IgG binding capacity. At a pH of 4.2 the highest binding capacity for polyclonal IgG is reached at a sodium chloride concentration of 300 mmol/L.

Post Protein A aggregate removal is a demanding step in downstream processing of monoclonal antibodies and cation exchange chromatography is the most popular mode applied for this purpose. TOYOPEARL Sulfate-650F was developed to provide a good resolution between mAb monomers and aggregates. Figure 2 proves the superior separation power of the new resin compared to other cation exchange media on the market.

Besides its salt tolerant cation exchange properties, TOYOPEARL Sulfate-650F offers an additional feature, known from other sulfate type absorbers: a certain heparin like affinity towards blood factors. All in all, the new resin is a highly selective, salt tolerant and high capacity cation exchange resin for the capture and intermediate polishing of biomolecules. It offers chromatographers the ability to use mobile phases at physiological conditions without any loss of capacity or selectivity.

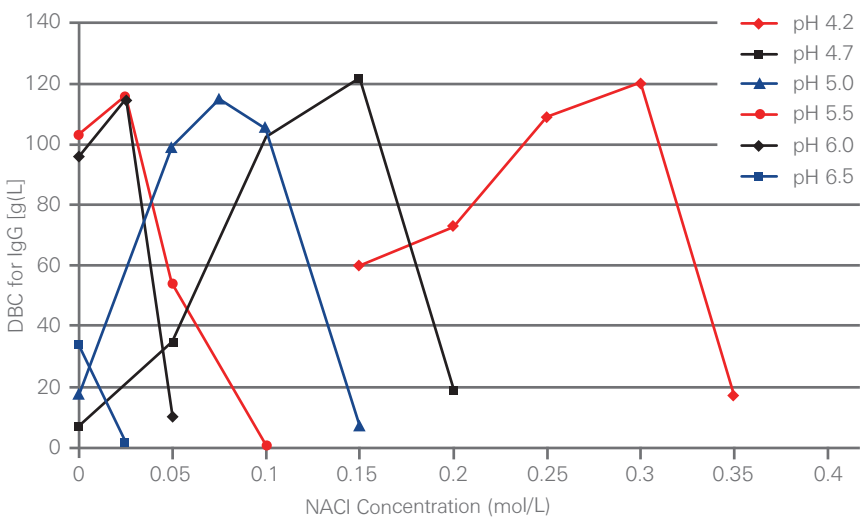

FIGURE 1: **EFFECT OF PH AND CONDUCTIVITY ON IgG DBC**

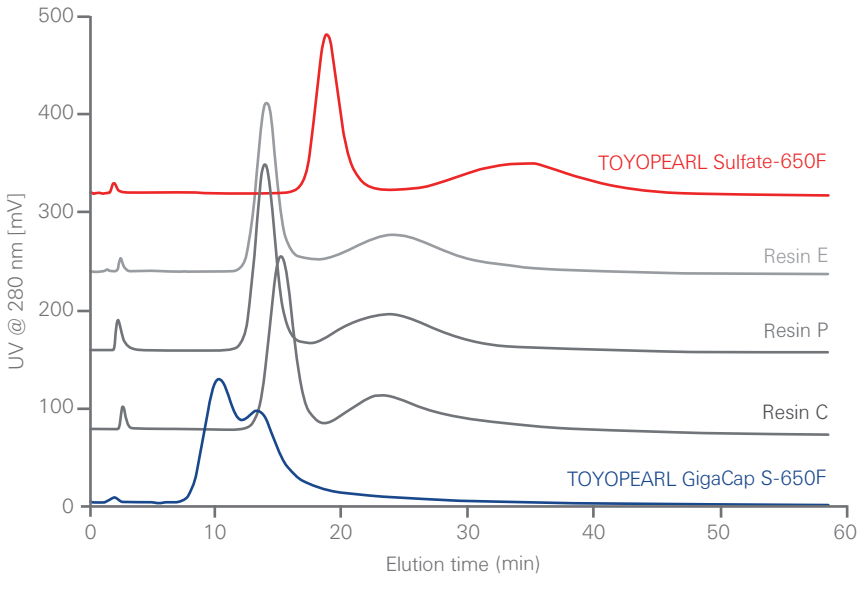

FIGURE 2: **AGGREGATE REMOVAL ON VARIOUS CATION EXCHANGE MEDIA AT PH 5.5**

07 GPC POLYMER ANALYSIS

REVEALING THE COMPLEXITY OF POLYMERS

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 molecular weight distribution of a polymer and its molecular weight averages play crucial roles in determining the mechanical, bulk and solution properties like processability, melt viscosity, tensile strength, impact resistance etc. Irrespective of the number of batch or reactor, a polymer is generally produced with identical polymerization conditions. Therefore, one can easily raise the question, what is the relevance of determining the molecular weight of the polymer from each batch and reactor produced under identical polymerization conditions? The answer to this question follows.

''We can never produce two identical polymer materials", stated by a senior polymer scientist from the industry, clearly expresses the requirement of evaluating the yield of a polymerization reaction, the quality of a polymer thus formed and the characteristics of the products made out of it.

Tosoh offers a dedicated GPC system, the EcoSEC®, for the determination of molecular weight and molecular weight distribution with extreme reproducibility in a shorter analysis time. Due to the compact design, EcoSEC provides stable temperature at the pump heads, column oven and RI detector which in return provides a stable RI baseline with extensively shorter time. Since we focus on the accuracy of the results, EcoSEC GPC systems are only designed to determine the molecular weight rather than using the instrument for other sophisticated chromatographic separations. We offer reproducible molecular weight results from day to day, system to system and locations to locations which allow the customers to compare their results from their worldwide subsidiaries. EcoSEC can perform the molecular weight analysis of a wide range of polymers in a variety of solvents.

EcoSEC is one of the GPC systems in the market with the lowest dead volume. However, reduced analysis time without compromising the resolution of the chromatogram was an issue until Tosoh developed its semi-micro columns. In addition, semi-micro columns reduce the consumption of solvent by 60 % which is a great environmental as well as economic advantage if we have to determine the molecular weight of polymers that dissolve only in expensive solvents such as hexafluoroisopropanol (HFIP).

The exchanging of columns consumes a lot of time and manpower. Tosoh considered this and developed a column switching valve for our customers which allow them to place two sets of 4 columns each that can basically cover the separation of all the range of molecular weights. The use of a column switching valve shortens the system shutdown and warm up time and saves up to 75 min (Figure1).

The complete solution for the determination of molecular weight of polymers, the all in one EcoSEC GPC systems including detectors, wide variety of highly stable GPC columns are designed and built by eminent Japanese engineers, tested by experienced polymer scientists and supported by the entire organization that you can trust and has a legacy of more than 40 years in this business under the powerful roof of Tosoh.

FOR POLYMER SAMPLE ANALYSES, INSTRUMENT / SOFTWARE DEMONSTRATIONS, AND OTHER RELATED QUESTIONS PLEASE CONTACT SUBIN.DAMODARAN@TOSOH.COM OR BERND.WOLF@TOSOH.COM

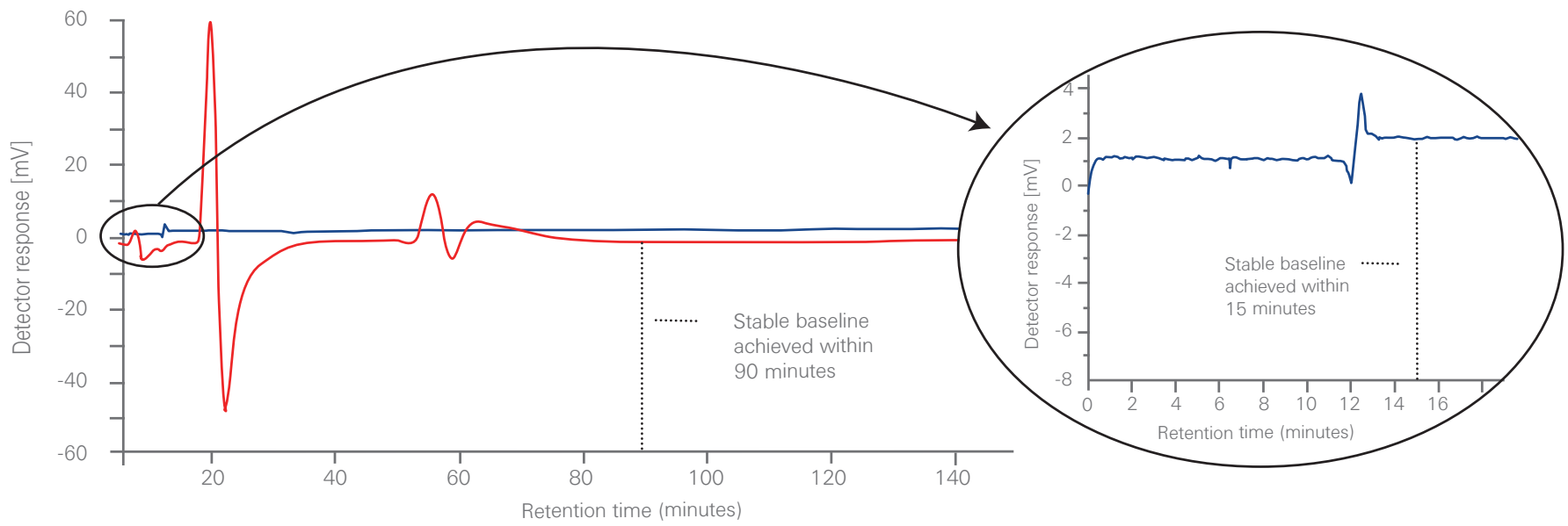

FIGURE 1: OVERLAY OF REFRACTIVE INDEX DETECTOR SIGNALS DURING EQUILIBRATION FOLLOWING A COLUMN CHANGE USING THE COLUMN SWITCHING VALVE (BLUE) AND WITHOUT USE OF THE COLUMNS SWITCHING VALVE (RED)

08 WHAT HAPPENED ANALYTICA

A LEAP OF FAITH AT THE ANALYTICA

GREENWASH (GRĒN'WŎSH', -WÔSH)[1] - USED TO DESCRIBE THE ACT OF MISLEADING CONSUMERS REGARDING THE ENVIRONMENTAL PRACTICES OF A COMPANY OR THE ENVIRONMENTAL BENEFITS OF A PRODUCT OR SERVICE.

After this rather provocative introduction, we want to share with you some thoughts about the organization of the ANALYTICA tradeshow in Munich earlier this year. On the impulsion of some Tosoh Bioscience colleagues, we decided to try and decrease as much as possible the environmental footprint of our participation in this event.

The first requirement was to keep the costs similar to 2014, and this is already our first success – the following changes could be implemented without major cost increases: Our booth structure – especially the back walls – is now designed to be reused at subsequent tradeshows. You will already find the same fabric panels at the ILMAC in Basel in September. Consumables have also been adapted to this philosophy – no coffee pads but whole beans, no plastic cups but reusable ones, organic beverages and food, fewer giveaways.

Last but not least, one huge CO_2-emission source of tradeshows is the transportation of the booth staff. Tosoh people were encouraged to reflect on their transportation habits – which was quite effective as over 60 % of the staff decided either to car pool or to use the train. Visitors who proved their travelling by train or bus got a special present to honor their contribution.

We do believe that these activities are no greenwash but a first step in the right direction, and we will continue to keep our marketing activities on this sustainability track. We would be excited if you could let us know your thoughts or even make proposals on how we could keep improving the sustainability of our actions. Drop us a message at info.tbg@tosoh.com or on Facebook!

[1]: **HTTP://WWW.STOPGREENWASH.ORG**

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES

UPCOMING EVENTS

SEP.	19 - 22	2016	KHIMIA 2016, FIZLABPRIBOR	MOSCOW [RUSSIAN FEDERATION]
SEP.	20 - 23	2016	ILMAC 2016	BASEL [SWITZERLAND]
SEP.	21 - 24	2016	16TH BALTIC POLYMER SYMPOSIUM, LABOCHEMA	KLAIPĖDA [LITHUANIA]
SEP.	26 - 29	2016	INTL. SYMPOSIUM ON GPC/SEC AND RELATED TECHNIQUES	AMSTERDAM [NETHERLANDS]
OCT.	4 - 7	2016	WORLD OF TECHNOLOGY AND SCIENCE, JSB	UTRECHT [NETHERLANDS]
NOV.	6 - 9	2016	ISPPP 2016	SALZBURG [AUSTRIA]
FEB.	7 - 8	2017	10TH BIOINNOVATION LEADERS SUMMIT, BILS 2017	BERLIN [GERMANY]
FEB.	12 - 16	2017	10TH HIC/RPC HYDROPHOBIC BIOPROCESSING CONFERENCE	SCOTTSDALE AZ [USA]
MARCH	20 - 23	2017	ARABLAB	DUBAI [UAE]

TRAININGS | WORKSHOPS

SEP.	20 - 22	2016	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION BASIC COURSE IN GERMAN LANGUAGE | STUTTGART | [GERMANY]
SEP.	27 - 29	2016	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION BASIC COURSE IN GERMAN LANGUAGE | STUTTGART | [GERMANY]
NOV.	22 - 23	2016	FORUM PROZESSCHROMATOGRAPHIE ADVANCED COURSE IN GERMAN LANGUAGE | GRIESHEIM | [GERMANY]

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