TOSOH

TOSOH
THE CUSTOMER
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NO. #02 2015

SEC / SEPARATION / SCHOLARSHIP

TOSOH BIOSCIENCE

02
EDITORIAL
DEAR READER

Dear reader, welcome to the second issue of the Tosoh Bioscience customer magazine in 2015. The motto of this issue is SEC/Separation/Scholarship. It is featuring our new UHPLC columns for size exclusion chromatography (SEC) of proteins, the new Tosoh scholarship for Students/PhD Students and Post Docs and several applications of our separation products in biopharma and chemical industry.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING I MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

THE SUPER-T - COMIC #2

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CONTENT

IMPRESSUM

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03
WHAT'S NEW COLUMNS

TSKgel UP-SW3000 COLUMNS FOR UHPLC USE

TSKgel UP-SW3000, THE NEW 2 MICRON UHPLC COLUMN WITH 25 NM PORE SIZE, IS THE LATEST ADDITION TO THE RENOWNED TSKgel SW SERIES OF SILICA BASED GEL FILTRATION COLUMNS. FOR DECADES, TSKgel G3000SWXL COLUMNS HAVE BEEN THE GOLD STANDARD FOR QC OF THERAPEUTIC ANTIBODIES IN BIOPHARMA. OVER 40 YEARS OF EXPERIENCE IN DEVELOPING AND PRODUCING SEC STATIONARY PHASES ARE BUILT INTO THE NEW SERIES OF UHPLC COLUMNS FOR THE ANALYSIS OF PROTEINS IN THE RANGE OF 10 TO 500 KDA.

TSKgel UP-SW3000 features the same pore size as the well-established TSKgel G3000SWXL. The calibration curve and the molecular weight range of the new 2 µm TSKgel UP-SW3000 is similar to those of 5 µm TSKgel G3000SWXL and 4 µm TSKgel SuperSW3000. Hence, methods developed on conventional gel filtration columns can be easily transferred to UHPLC technology by using TSKgel UP-SW.

TSKgel UP-SW is available in two column lengths; The shorter 15 cm column can be used to speed up analysis and increase throughput while maintaining resolution. The 30 cm column delivers dramatically increased resolution e.g. between mAb fragments, monomers, and aggregates. The use of the 15 cm column for aggregate analysis is described on page 5.

To achieve maximum resolution and fully exploit the potential of the columns the use of a UHPLC system is highly recommended. A so-called DC (direct connect) guard column can be attached directly to the analytical column to minimize extra column band broadening while preventing early deterioration of the analytical column.

TSKgel SW columns stand out from other silica-based high performance size exclusion columns by virtue of their large pore volumes. They are based on highly porous, ultra-pure silica particles, the surface of which has been shielded from interacting with proteins by applying a proprietary surface chemistry. This ensures highest recoveries and accurate quantitation. The validated manufacturing and packing process delivers a reliable batch-to-batch reproducibility.

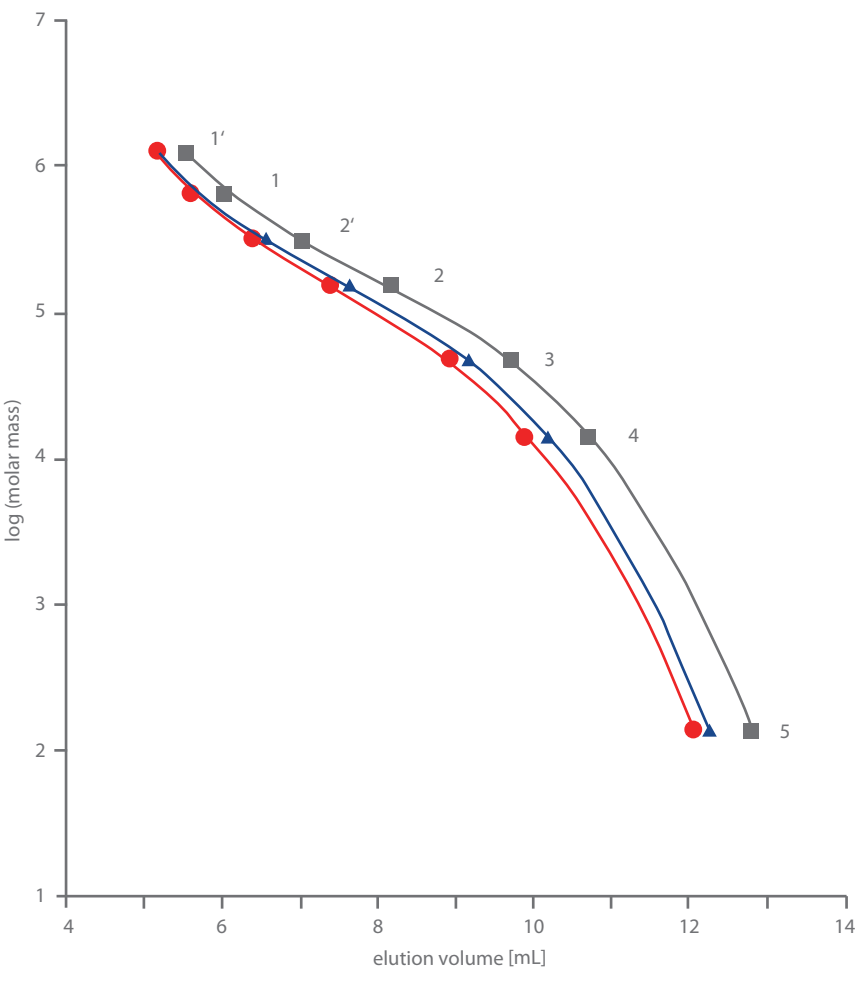


FIGURE 1 **COMPARISON OF CALIBRATION CURVES PROTEIN STANDARD ON TSKgel UP-SW3000 (RED), TSKgel SuperSW3000 (BLUE) AND TSKgel G3000SWXL (GREY)**

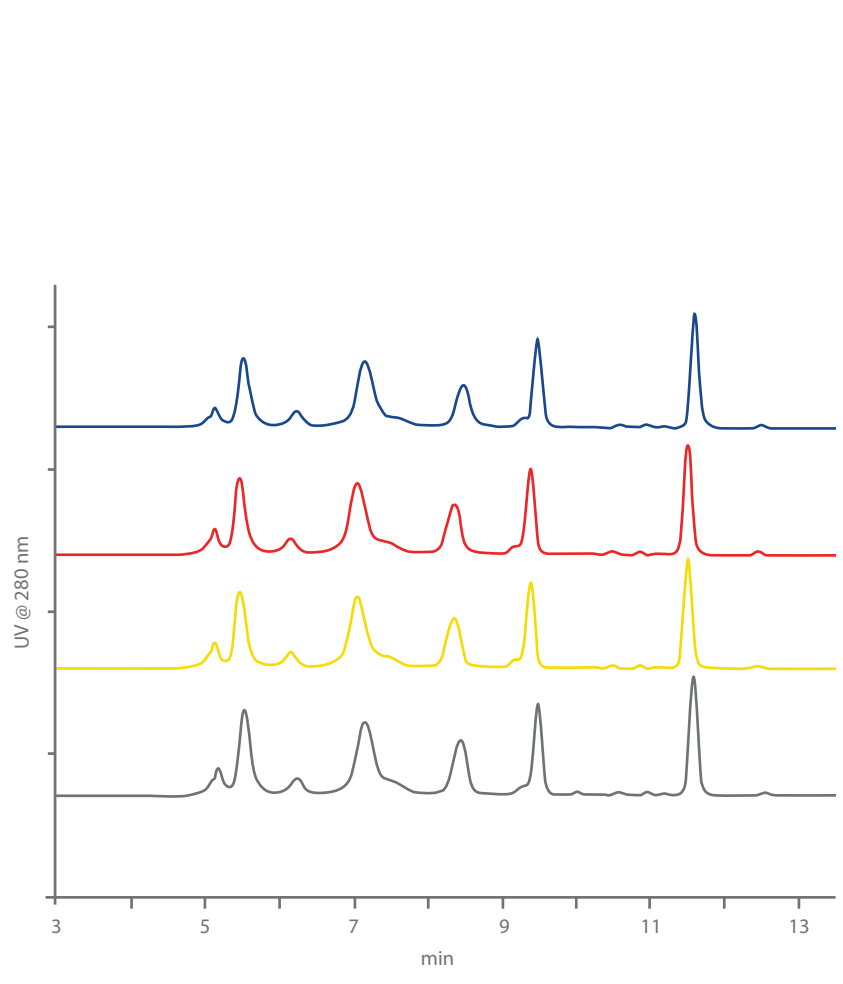


FIGURE : **BATCH-TO-BATCH REPRODUCIBILITY FOR TSKgel UP-SW3000**

04 APPLICATION MONOCLONALS I

MAB AGGREGATE REMOVAL BY SALT-TOLERANT ION EXCHANGE CHROMATOGRAPHY

PURIFICATION SCHEMES FOR MONOCLONAL ANTIBODIES TYPICALLY CONSIST OF THREE CHROMATOGRAPHIC STEPS ACCOMPANIED BY FILTRATION STEPS. THE COMMON PROTEIN A CAPTURING IS TYPICALLY FOLLOWED BY ION EXCHANGE (IEC), HYDROPHOBIC INTERACTION (HIC), OR MIXED-MODE POLISHING STEPS. RESIDUAL DNA, VIRUSES, AND HOST CELL PROTEINS ARE USUALLY REMOVED BY FLOW-THROUGH ANION EXCHANGE CHROMATOGRAPHY WHILE AGGREGATES CAN BE REDUCED THROUGH A CATION EXCHANGE, MIXED-MODE, OR HIC STEP.

The salt tolerant anion exchange resin TOYOPEARL NH2-750F provides a unique selectivity compared to other anion exchange resins and was also found to be suited for aggregate removal. In general, anion exchange resins can be used in bind and elute (B/E) mode as well as in flow-through (FT) mode. Both options were evaluated for TOYOPEARL NH2-750F. To increase the amount of aggregates of the test sample, a monoclonal antibody was aggregated by acidic incubation and subsequently diluted to 1 g/L in loading buffer.

The dynamic binding capacity of TOYOPEARL NH2-750F for the mAb used in this study was evaluated and a value of 95 mg/mL could be reached with 10 mM Tris/HCl, pH 8.0 for B/E mode. SEC analyses of fractions of the elution profile of the aggregated antibody on TOYOPEARL NH2-750F show that aggregates do not elute in the salt gradient and remain bound until the column is CIPed with sodium hydroxide. Fractions 10 to 14 have an aggregate content below the limit of detection of SEC.

In order to establish a FT polishing step, buffer conditions were evaluated to optimize non-binding conditions for the monomer by varying pH and salt content. Best results were obtained with 10 mM Tris/HCl, pH 7.0 at a sodium chloride concentration of 250 mM. To analyze the aggregate removal, 100 mg aggregated antibody were loaded on a 2 mL column and fractions of the flow through were analyzed by SEC. All FT fractions are essentially aggregate free.

TOYOPEARL NH2-750F is ideally suited to develop a polishing step for monoclonal antibody by either using the resin in BE mode or in FT mode. For both modes ideal conditions for aggregate removal could be established. An additional benefit when using this resin in FT mode is the delivered excellent viral clearance. Typical virus log reduction exceeds five for enveloped and non-enveloped DNA and RNA viruses.

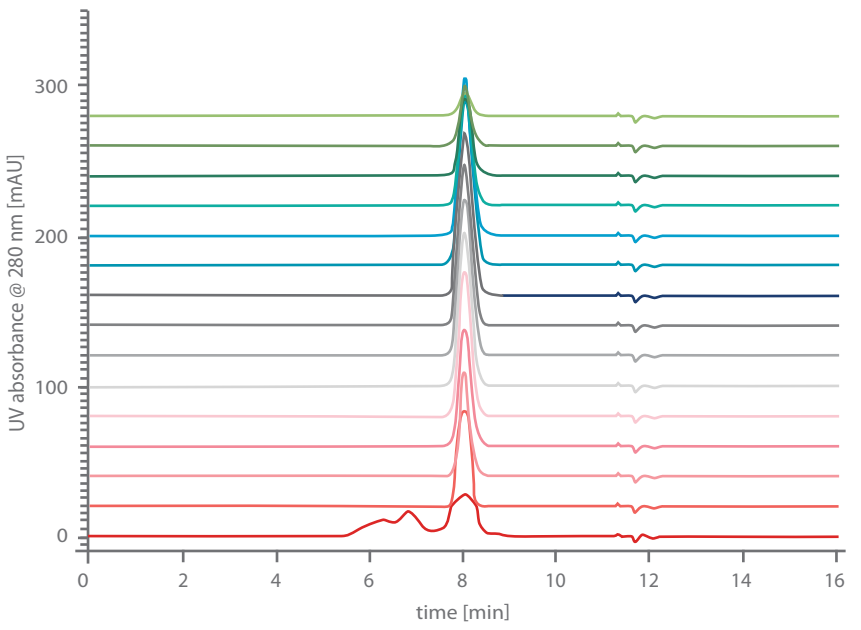
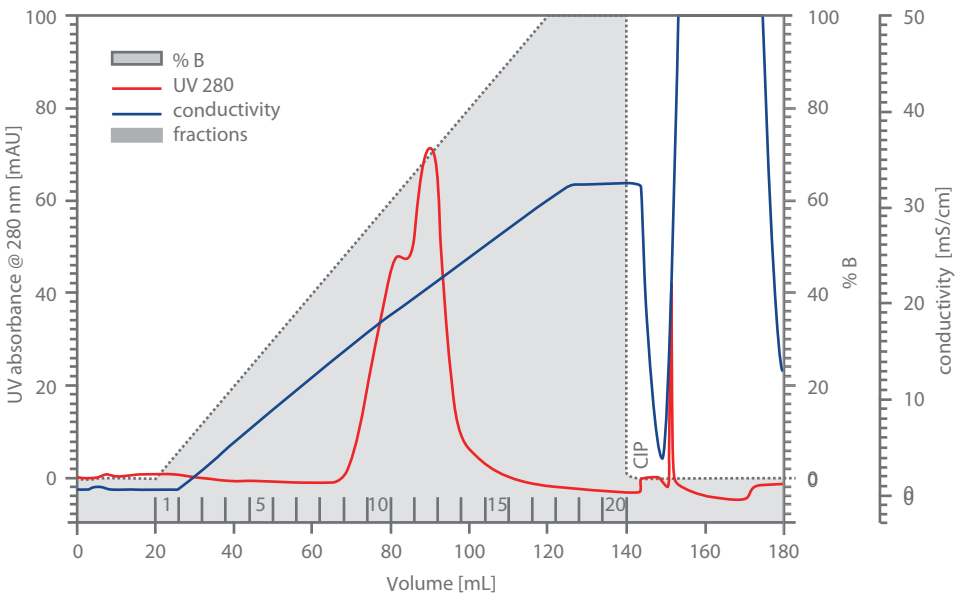


FIGURE 2 : **SEC ANALYSIS OF THE AGGREGATED mAb SAMPLE (RED LINE AT 0 MAU) AND FLOW-THROUGH FRACTIONS IN INCREASING FRACTION ORDER FROM BOTTOM TO TOP.**



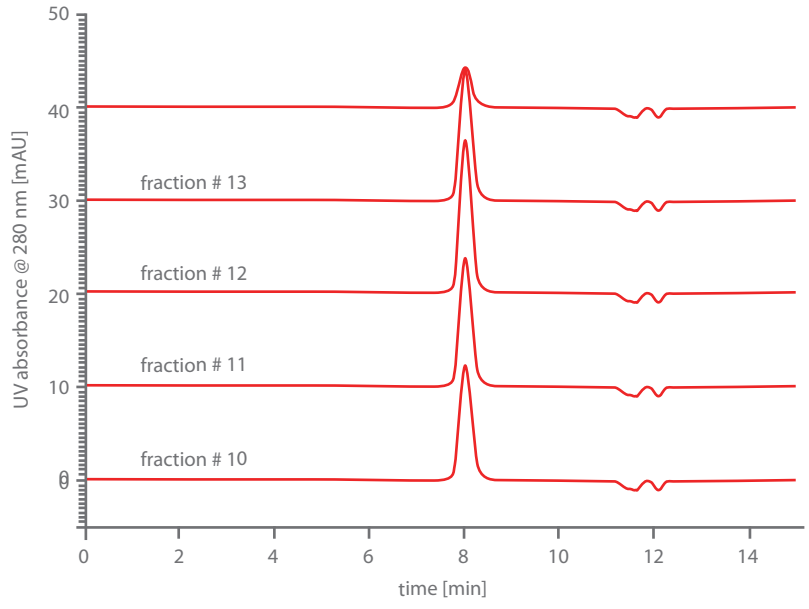


FIGURE 1 **ELUTION PROFILE OF AGGREGATED ANTIBODY ON TOYOPEARL NH2-750F IN B/E MODE AND ANALYSIS OF FRACTIONS BY SEC ON TSKgel G3000SWXL**

05 APPLICATION MONOCLONALS II

MAB AGGREGATE ANALYSIS BY UHPLC

ANTIBODY THERAPEUTICS ARE ENJOYING HIGH GROWTH RATES. IN 2013, SIX OF THE TOP TEN BEST-SELLING GLOBAL DRUG BRANDS WERE MONOCLONAL ANTIBODIES (MABS) AND MORE THAN 400 MONOCLONALS WERE IN CLINICAL TRIALS. THE CHARACTERIZATION OF THESE COMPLEX BIOMOLECULES IS A MAJOR CHALLENGE IN PROCESS MONITORING AND QUALITY CONTROL. THE MAIN PRODUCT CHARACTERISTICS TO BE MONITORED ARE AGGREGATE AND FRAGMENT CONTENT, GLYCOSYLATION PATTERN AND CHARGED ISO-FORMS.

The standard method used in biopharmaceutical QC for mAb aggregate and fragment analysis is size exclusion chromatography (SEC). A new series of 2 micron silica based UHPLC columns with 25 nm (250 Å) pore size can be applied to either increase speed or improve resolution of the separation of antibody fragments, monomers, and dimers.

Compared to a commercially available 1.7 micron UHPLC column the calibration curves of the new TSKgel UP-SW3000 2 µm column (see page 3) shows a slightly shallower slope in the region of the molecular weight of γ-globulin. These differences in the separation range and steepness of the curves are related to a slight difference in pore size (25 nm for TSKgel versus 20 nm for the 1.7 µm material).

The separation of an antibody sample on the new 2 µm packing compared to the competitor UHPLC column shows that the small difference in pore sizes results in a better separation in the molecular weight range of antibodies, fragments, and aggregates. Due to the wider separation window the resolution between monomer and dimer as well as dimer and trimer is slightly higher with TSKgel UP-SW3000 although particle size is slightly larger than in the competitor column. Moreover, also the fragment peak is more clearly separated from the monomer peak.

TSKgel UP-SW3000 is ideally suited for the analysis of the aggregate and fragment contents of antibody preparations. It features the same pore size as the renowned TSKgel G3000SWXL and TSKgel Super mAb columns while improving resolution through a smaller particle size. Based on the optimized pore size and the high degree of porosity the resolution in the molecular weight range of immunoglobulins is even superior to a competitive UHPLC column with slightly smaller particle and pore size.

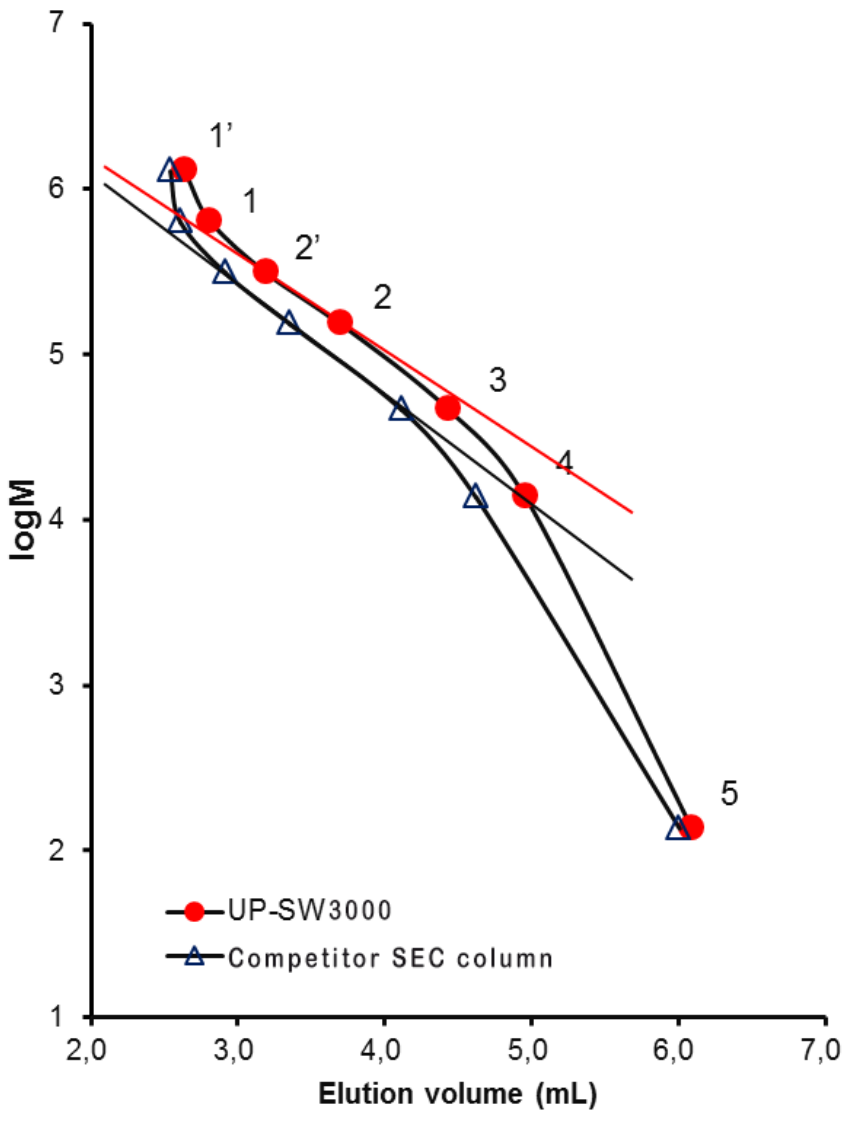


FIGURE 1 **CALIBRATION CURVES**
PROTEIN STANDARD ON 2 µMY TSKgel UP-SW3000 AND 1.7 µMY COMPETITOR COLUMN

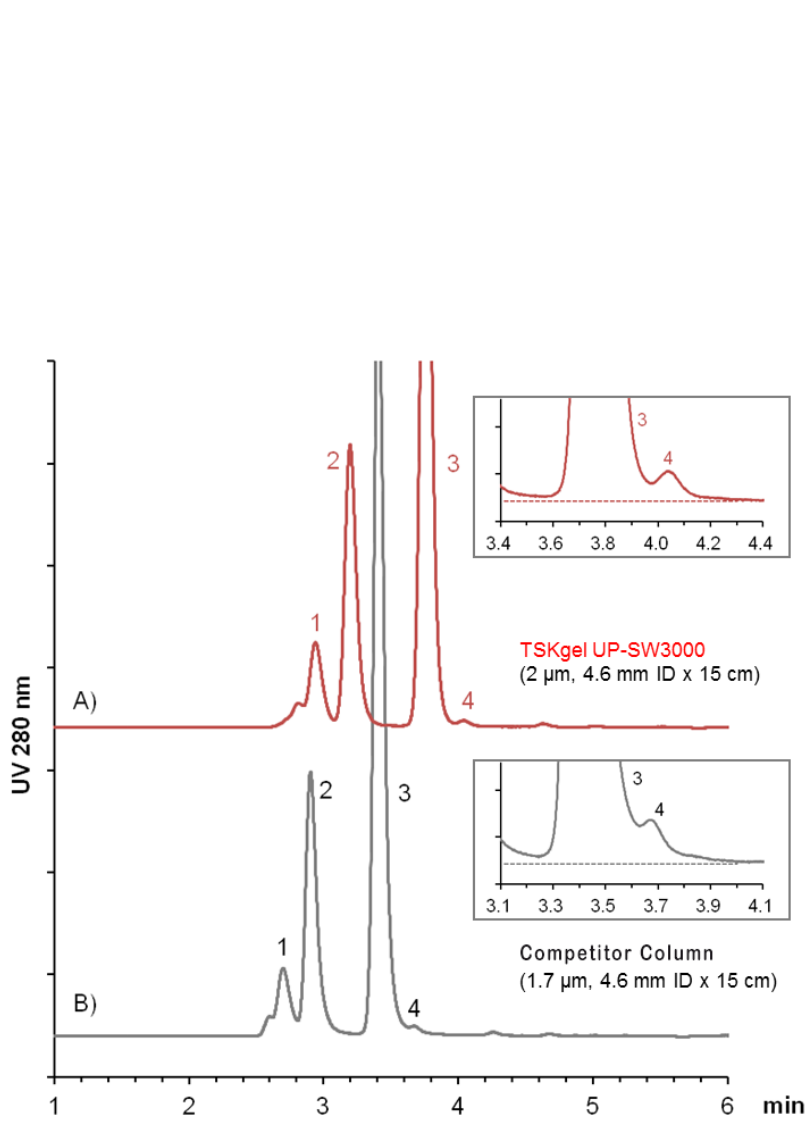


FIGURE 2 : **COMPARISON OF ANTIBODY ANALYSIS RESULTS**
MOUSE-HUMAN CHIMERIC mAb; 1: TRIMER; 2: DIMER; 3: MONOMER ; 4: FRAGMENT

06 TOSOH SCHOLARSHIP

AND THE WINNER IS: JOSEFINE MORGENSTERN FROM KIT, KARLSRUHE

AT HPLC 2015 IN GENEVA TOSOH BIOSCIENCE PRESENTED THE NEW TOSOH CHROMATOGRAPHY SCHOLARSHIP. FOR THIS FIRST EDITION MASTER AND PHD STUDENTS AS WELL AS POST-DOCS HAD THE OPPORTUNITY TO SUBMIT PROJECTS ON PURIFICATION OF BIOMOLECULES. OUT OF THE MANY APPLICATIONS SUBMITTED, MS. JOSEFINE MORGENSTERN WAS APPOINTED AS THE WINNER OF THE SCHOLARSHIP. IT CONSISTS OF A FREE PARTICIPATION IN THE RENOWNED TOSOH BIOSCIENCE CHROMATOGRAPHY TRAINING IN DOWNSTREAM PROCESSING AND A STARTER PACKAGE OF CHROMATOGRAPHY MEDIA NEEDED FOR THE PROPOSED PROJECT. THE SCHOLARSHIP WAS OPEN TO GERMAN-SPEAKING STUDENTS AS THIS WAS THE LANGUAGE OF ALL THREE WORKSHOPS IN 2015.

The projects submitted for the scholarship covered a broad range of target molecules: from enzymes from recombinant and natural sources over ingredients of citrus fruits to biofilms. Josefine – a Ph.D. student at the Institute of Process Engineering in Life Sciences, Biomolecular Separation Engineering (MAB), Karlsruhe Institute for Technology – proposed a project on purification of PEGylated proteins. Her final target is to analyze the influence of polymer modifications on the stability of proteins used in industrial applications.

The selected modification – called PEGylation – consists in the covalent attachment of polyethylene glycol (PEG) to the target molecule. A typical PEGylation reaction delivers a mixture of products that differ either in the number of bound PEG chains (PEGamers) or in the position of bound PEG molecules (isoforms). The PEGamers and isoforms need to be separated in order to analyze their individual stability.

Liquid chromatography already proved to be a good approach to separate PEGylated proteins, for preparative but also for analytical purposes. Ion exchange and size exclusion chromatography will be applied in Josefine's project first and we are happy to supply a portfolio of TOYOPEARL GigaCap ion exchange media and HW size exclusion media for this project. Chromatography experts from Tosoh Bioscience will support Josefine throughout her projects through periodic consultancy.

The prize and the certificate were handed over at the Chromatography Workshop held in Stuttgart in September 2015.

THE TOSOH GRANT 2016 **WILL SUPPORT AN ANALYTICAL APPLICATION OF BIOCHROMATOGRAPHY.**

07 TSKgel/EcoSEC IN THE LITERATURE

OF SILVER/POLYMER NANOCOMPOSITES AND ARMED ANTIBODIES

OUR CHROMATOGRAPHY COLUMN, MEDIA, AND INSTRUMENT BRANDS - TSKgel, TOYOPEARL, AND EcoSEC - ARE FREQUENTLY MENTIONED IN SCIENTIFIC ARTICLES. TODAY WE PICKED TWO VERY INTERESTING EXAMPLES PUBLISHED THIS YEAR. THEY PRESENT RESULTS IN TWO REALLY DIVERGING FIELDS OF RESEARCH AND APPLICATION: POLYMER CHARACTERIZATION ON ONE SITE AND RESEARCH ON ARMED ANTIBODIES ON THE OTHER SITE.

Yifan Pang and coworkers applied the EcoSEC compact GPC system to analyze AG/Polymer Nanaocomposites[1]. Nanocomposite materials consist of polymer and inorganic nanoparticles (NPs) especially noble metal NPs have shown valuable application in various fields. One example is the antibacterial grafting of cotton materials. Of them Nanocomposites containing silver NPs have received intensive research interest for its unique features in the fields of catalysis, antimicrobial agents, conducting materials and sensors.

The authors describe a novel synthetic strategy for microspheres and micro-plates of Ag/POA nanocomposites in the absence of solvent and surfactant. The method combines the nucleation of Ag nanoparticles and the polymerization of monomer in a facile one-pot reaction, which provides a novel way for metal-polymer microsphere nanocomposite with low-cost, easy-operation and high-yield. The molecular weights of the products were measured by GPC on a EcoSEC GPC System system at 40 °C with THF as mobile phase.

Dario Venetz and coauthors used TSKgel HILIC columns to analyze the Glycosylation profile of armed antibodies [2]. Therapeutic antibodies represent the largest and fastest growing class of biopharmaceuticals. There is a trend in moving from intact antibodies towards “armed” antibody products, in which the antibody moiety serves as pharmacodelivery vehicle. The impact of glycosylation on the targeting performance of armed antibodies is still largely unknown.

The article sheds light on the surprising finding that relatively small variations in glycostructures and sialic acid content can have dramatic effects on therapeutic agent performance. By performing detailed biodistribution studies with a novel IL9-armed cancer-specific antibody, the authors identified a clear correlation between N-linked glycan structures and tumor-targeting efficiencies.

[1] PANG, Y. ET AL. UNEXPECTED IN-SITU FREE RADICAL GENERATION AND CATALYSIS TO AG/POLYMER NANOCOMPOSITE. SCI. REP. 5, 11993; DOI: 10.1038/SREP11993 (2015).

[2] D. VENETZ, ET AL. GLYCOSYLATION PROFILES DETERMINE EXTRAVASATION AND DISEASE-TARGETING PROPERTIES OF ARMED ANTIBODIES, PNAS 112.7: 2000-2005; 10.1073/PNAS1416694112 (2015)

08
WHAT'S HAPPENING
WEBINAR

CURRENT TRENDS IN THE ANALYSIS OF BIOLOGICS, BIOSIMILARS & BIOBETTERS

DEVELOPMENT AND PRODUCTION OF BIOPHARMACEUTICALS IS A GROWING SEGMENT OF PHARMACEUTICAL INDUSTRY. A THOROUGH CHARACTERIZATION OF THERAPEUTIC BIOMOLECULES IS A KEY FOR THE SUCCESSFUL SUBMISSION OF RESEARCH AND PRODUCTION DATA FOR REGULATORY APPROVAL OF NEW DRUGS. THE INTRODUCTION OF THE FIRST SO-CALLED BIOSIMILARS HAS FURTHER INCREASED THE DEMAND FOR HIGHLY EFFICIENT ANALYTICAL METHODS. TOSOH BIOSCIENCE WILL HOST A WEBINAR ON THIS TOPIC ON NOVEMBER 12, 2015.

Over the last decade, ultrahigh-performance liquid chromatography (UHPLC) has made its way into routine labs for the analysis of small molecules and active pharmaceutical ingredients (APIs). It has triggered a leap in efficiency and throughput of LC and LC-MS methods and there is an increased interest in applying UHPLC also to biopharmaceutical analysis.

The analysis of the aggregate content and charge heterogeneity are typical applications applied to characterize therapeutic proteins, such as monoclonals. Smaller peptides or the oligosaccharides of the glycan structure of a protein are typically analysed after enzymatic cleavage. Recently, the analysis of antibody drug conjugates (ADCs), bispecific antibodies, and therapeutic oligonucleotides by chromatographic methods gained attention to characterise these new drug formats.

The webinar will summarize current trends in UHPLC applications for biomolecules. Case studies will illustrate the potential of using UHPLC for applications, which are frequently used in development and production of biologics, biosimilars, and biobetters. New bio-column developments supporting these techniques will be presented. Hyphenation of these LC Methods with sophisticated techniques such as mass spectrometric, light scattering, or Raman detection will also be covered.

➨ **REGISTER FOR THE WEBINAR AT** HTTP://BIT.LY/1M4YPRN

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES

UPCOMING EVENTS

➨	FEB. 10 - 11	| 2016	➨	9TH ANNUAL BIO INNOVATION LEADERS SUMMIT | BERLIN [GERMANY]
➨	FEB. 23 - 24	| 2016	➨	DOWNSTREAM PROCESSING WORLD 2016 | MUNICH [GERMANY]
➨	FEB. 24 - 26	| 2016	➨	MACROMOLECULAR COLLOQUIUM | FREIBURG [GERMANY]
➨	MAR. 20 - 23	| 2016	➨	ARABLAB | DUBAI [UNITED ARAB EMIRATES]
➨	APRIL 12 - 13	| 2016	➨	BIOPROCESS INTERNATIONAL EUROPE | VIENNA [AUSTRIA]
➨	MAY 10 - 13	| 2016	➨	ANALYTICA 2016 | MUNICH [GERMANY]

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