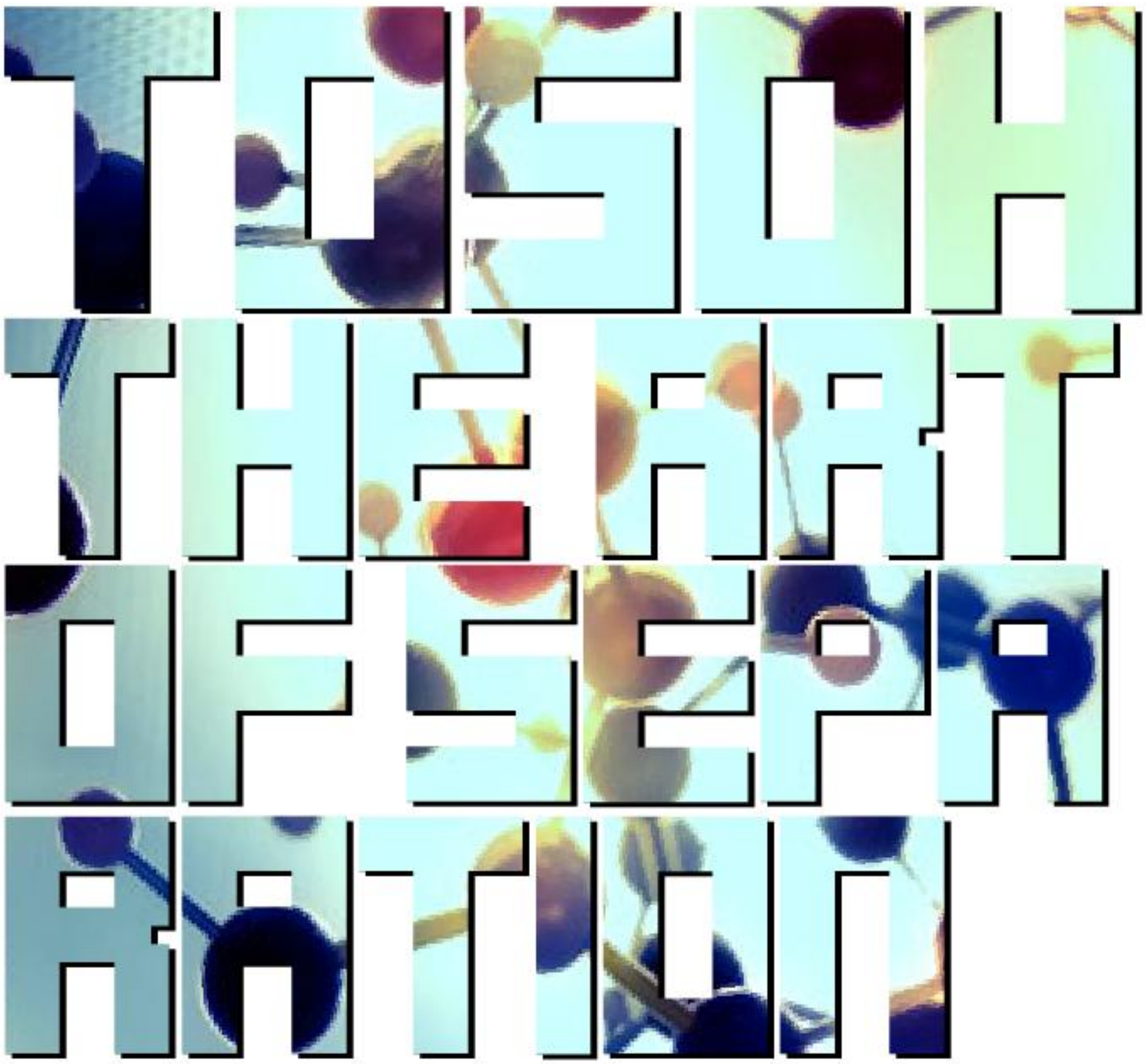
TOSOH

TOSOH
THE CUSTOMER
MAGAZINE

NO.#01 2016

ANALYTICA / APPLICATION / ACCESS



TOSOH BIOSCIENCE

02
EDITORIAL
DEAR READER

Dear reader, welcome to the spring 2016 issue of the Tosoh Bioscience customer magazine. From May 10 to 13, analytica 2016 and the analytica conference will offer visitors the opportunity to gather information on the latest products and scientific trends. We invite you to visit our booth (A2.410) to discuss your separation needs with our specialists and check out the news from Tosoh. This year, the Tosoh Scholarship for Chromatography will cover HPLC/UHPLC applications for biomolecules and will be open to applications at analytica.

The applications presented in this issue of the customer magazine cover new options for sanitization of Protein A media and high temperature gel permeation chromatography. We further facilitated the access to information about our solutions and services: our website got a new and modern layout, we spread our news now also on LinkedIn and not only on Facebook, and last but not least a new clip provides an easy introduction to liquid chromatography in general.

ENJOY READING AND STAY INFORMED.

REGINA ROEMLING I MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

CONTENT

IMPRESSUM

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03
WHAT'S NEW ONLINE

FROM PRESENTING TO NETWORKING – MEET TOSOH BIOSCIENCE IN THE WEB

SINCE THE FOUNDATION OF TOSOH BIOSCIENCE GMBH IN 1989 COMMUNICATION MODELS HAVE EVOLVED AT AN ACCELERATING PACE. TRADITIONAL MEDIA SUCH AS PRINTED MAGAZINES AND BROCHURES REMAIN A TOOL FOR SPREADING INFORMATION BUT EMAILING AND THE INTERNET ARE EVEN MORE DOMINANT WAYS OF INTERACTING WITH OUR CUSTOMERS. TODAY, MOBILE DEVICES AND SOCIAL MEDIA LIKE FACEBOOK, YOUTUBE OR LINKEDIN ARE POPULAR WAYS OF GATHERING INFORMATION. TOSOH IS CONTINUOUSLY EXPANDING THE OFFERINGS TO COVER ALL RELEVANT CHANNELS. HERE IS A SUMMARY OF THE LATEST DEVELOPMENTS OF OUR ONLINE PRESENCE.

In February 2016, we relaunched our website www.tosohbioscience.de using the new global CMS platform of Tosoh Corporation. Relevant news and product information are now presented in a fresh and modern look. Popular parts such as the technical support section including explanations of the various chromatographic modes are structured similar to the previous website to facilitate the usage of the new site. The website will be further developed to provide more information related to specific markets.

Today social media provide easy ways to be linked to the world and to each other. Tosoh Bioscience started marketing activities in social media a couple of years ago. The first difficulty was to pick out the right channel, as new solutions just pop up every day. The first choices were Twitter, for instant blogging, and Facebook, for more casual information about the daily life at Tosoh Bioscience GmbH such as invitation to events, pictures thereof or general information about our company or products. Starting communicating on Facebook was one of the most fruitful decisions. With over 10.000 followers, the number one social network has also become the number one social media channel for the communication of Tosoh Bioscience GmbH.

Until very recently, we could not use the most prominent professional network, LinkedIn due to technical issues. Now these were resolved and we started using LinkedIn intensively. We expect this platform to become a major tool for direct communications with professionals in academia and industry for Tosoh Bioscience GmbH. In parallel to social networking, our YouTube channel with both traditional videos as well as lighthearted cartoons is growing and very successful with over 70.000 views. The clips became very popular not only among the initial target audience of students and young professionals. We got very positive feedback also from 'old hands' in chromatography and some of the videos are even used for training purposes at universities.

Another tool - launched last year - is our mobile app for both iOS and Android smartphones. This app is the perfect example of combining all kinds of marketing activities: traditional contact methods such as email and phone numbers of all contact persons within EMEA, traditional communication tools such as PDFs of brochures and catalogs, and Facebook and YouTube feeds.

THE SUPER-T - COMIC #3

MEET ME AT THE "ANALYTICA" IN MUNICH, MAY 10-13, HALL A2, BOOTH 410

04
APPLICATION
DOWNSTREAM PROCESSING

THERMO-SANITIZATION OF PROTEIN A CHROMATOGRAPHY RESINS

PROTEIN A CHROMATOGRAPHY IS A WIDELY APPLIED PURIFICATION PLATFORM FOR CAPTURING OF MONOCLONAL ANTIBODIES. COMMON CLEANING IN PLACE STRATEGIES OFTEN INVOLVE SODIUM HYDROXIDE TREATMENT OF THE RESIN. STABILITY OF NATURAL PROTEIN A LIGANDS AGAINST SODIUM HYDROXIDE IS LOWER COMPARED TO LIGANDS USED IN HYDROPHOBIC INTERACTION OR ION EXCHANGE CHROMATOGRAPHY. EVEN THOUGH INTENSE EFFORTS HAVE BEEN INVESTED TO GENERATE ALKALINE STABLE PROTEIN A LIGANDS, BACTERIAL GROWTH IN PACKED COLUMNS MAY REMAIN AN ISSUE. HEREIN, WE DESCRIBE AN ALTERNATIVE APPROACH FOR CLEANING IN PLACE, WHICH IS BASED ON HEAT TREATMENT OF THE RESIN.

Heat treatment has been applied for decades to prevent bacterial growth in the beverage and food industry. Static and dynamic binding capacity of TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F is not affected by 30 minutes wet incubation at 90 °C. The host cell protein removal of these resins after thermo-treatment is similar.

The success story of protein A chromatography as a capturing step in mAb production has been pushed by the introduction of alkaline stable protein A ligands. The TOYOPEARL Protein A resin family has two alkaline stable members, namely TOYOPEARL AF-rProtein A-650F and TOYOPEARL AF-rProtein A HC-650F, 'HC' standing for high capacity. 80 % of the dynamic binding capacity (DBC) of TOYOPEARL AF-rProtein A HC-650F is preserved after 300 cycles of treatment with 0.2 M sodium hydroxide at a contact time of 15 minutes (Figure 1).

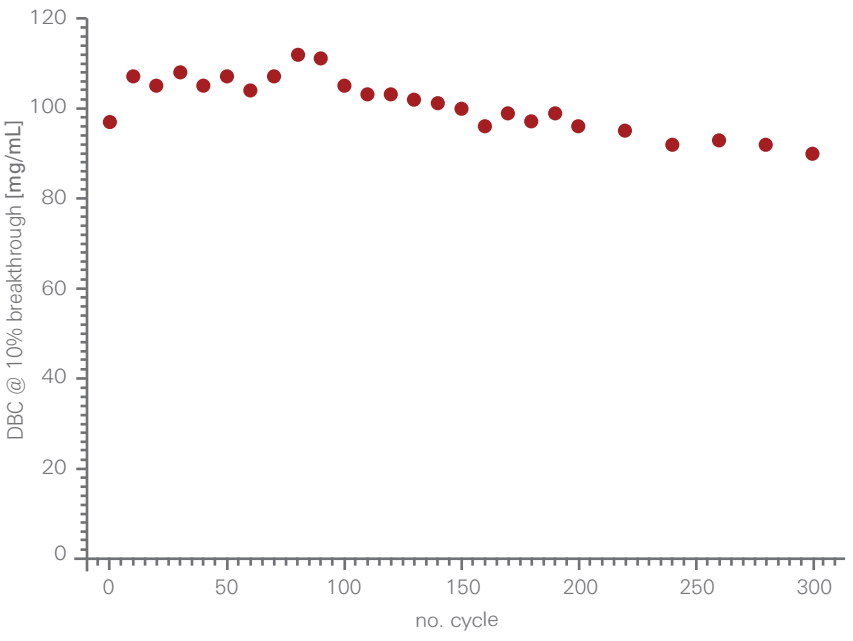


FIGURE 1 **DBC OF TOYOPEARL AF-rProtein A HC-650F FOR mAb. THE RESIN WAS TREATED WITH 0.2 M NaOH AT A CONTACT TIME OF 15 MIN. DBC WAS DETERMINED WITH A 5 G/L mAb SOLUTION AT 5 MIN RESIDENCE TIME IN 100 mM SODIUM PHOSPHATE BUFFER, pH 6.5.**

maceutical production. Tanks and other installations that hold water for injection (WFI) require sanitization, as well. According to the US Pharmacopeia, "temperatures of at least 80 °C are most commonly used for this purpose" [2]. The World Health Organization recommends in its Specifications for Pharmaceutical Preparations periodical sanitization of water distribution pipework with temperatures exceeding 70 °C to inhibit growth of microorganisms [3]. Thus, use of thermal sanitization for chromatography resins seems a promising alternative to chemical sanitization.

TOYOPEARL AF-rProtein A HC-650F has been incubated in aqueous solution at 90 °C for 30 minutes. The thermo-treated resin has been used in static binding capacity experiments. No significant change in static binding capacity can be observed (Figure 2).

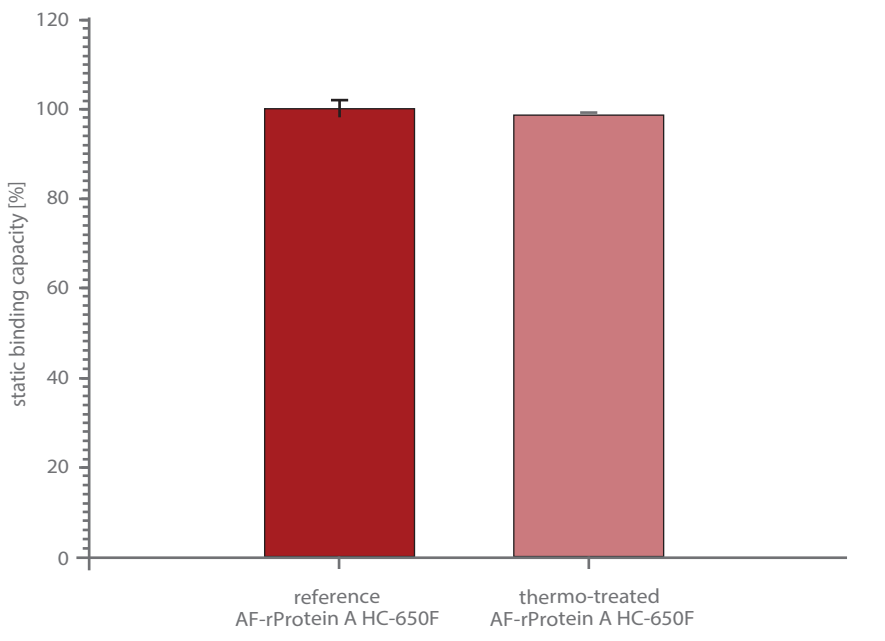
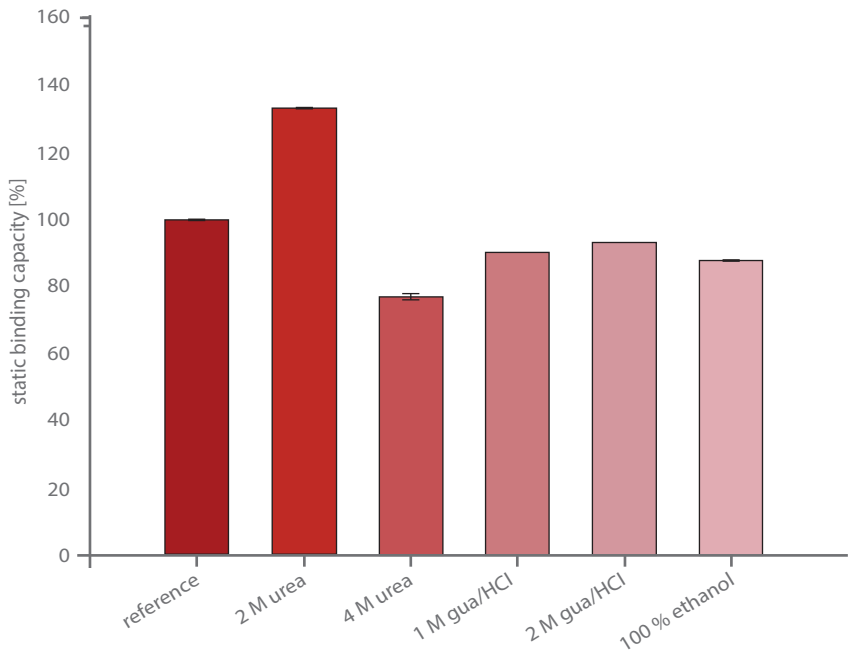


FIGURE 2 : **STATIC BINDING CAPACITY OF TOYOPEARL AF-rProtein A HC-650F AFTER THERMO-TREATMENT AND WITHOUT THERMO-TREATMENT. THE STATIC BINDING CAPACITY OF THE RESIN FOR mAb IS NOT SIGNIFICANTLY AFFECTED.**

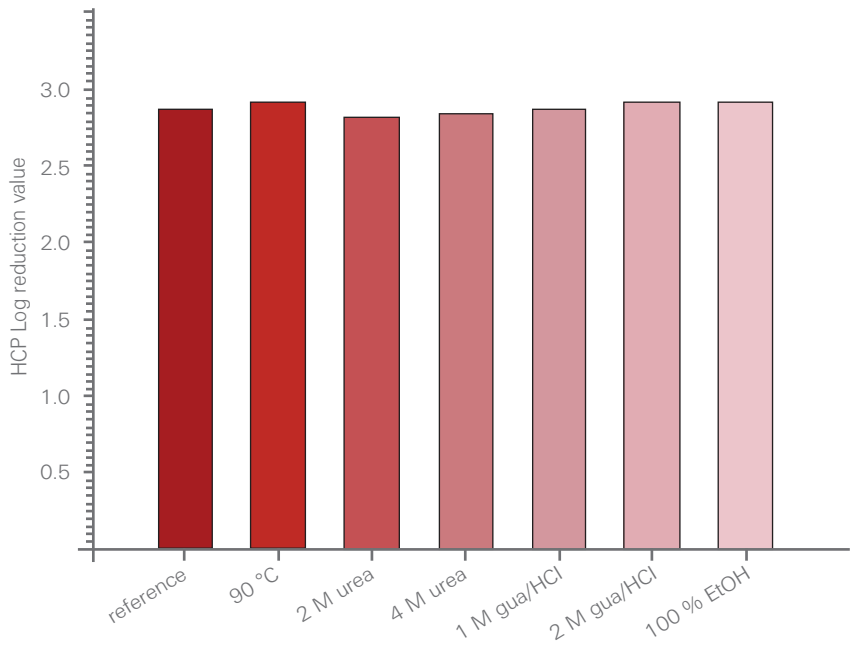
Alternative agents or treatments frequently applied in the biopharmaceutical industry include chaotropic agents, such as guanidinium hydrochloride or urea. However, other industry sectors, such as the food and beverage industry sector face similar challenges with regards to bacterial contamination. Wet heat treatment is one approach that is applied for the sanitization of bottling plants. Wet heat treatment at 77 °C (170 °F) for at least 15 minutes or at 93 °C (200 °F) for at least 5 minutes is recommended by the authorities [1]. Thermal sanitization can also be applied in biophar-

The impact of chaotropic agents, such as guanidinium hydrochloride and urea on static binding capacity of TOYOPEARL AF-rProtein A HC-650F has been investigated. Aliquots of the resin have been incubated for 70 hours in solutions of these agents at different concentrations. Static binding capacities are shown in Figure 3.



➨ **FIGURE 3 : STATIC BINDING CAPACITY OF TOYOPEARL AF-rProtein A HC-650F AFTER 70 HOURS INCUBATION IN VARIOUS DIFFERENT CHAOTROPIC SOLUTIONS. INCUBATION IN 2 M UREA INCREASES STATIC BINDING CAPACITY. GUANIDINIUM HYDROCHLORIDE OR 100 % ETHANOL HAVE MINOR IMPACT ON STATIC BINDING CAPACITY OF THE RESIN.**

Thermo-treated resin and resin that has been incubated with chaotropic agents were packed into 6.6 mm ID x 5 cm L columns. MAb feedstock was purified with thermo-treated resin and the protein A elution pool was analyzed with regards to their host cell protein (HCP) content. HCP titers were determined using CHO HCP ELISA kit 3G (Cygnus Technologies). TOYOPEARL AF-rProtein A HC-650F resin treated with sodium phosphate buffer at room temperature served as a reference. Log reduction values are roughly 2.9 for all of the tested conditions. No significant decrease in HCP removal after thermo-treatment or incubation with chaotropic agents is observed (Figure 4).



➨ **FIGURE 4 : LOG REDUCTION VALUES FOR HOST CELL PROTEIN REMOVAL. THE REFERENCE SAMPLE HAS BEEN TREATED WITH SODIUM PHOSPHATE BUFFER AT ROOM TEMPERATURE. NEITHER THERMO-TREATMENT NOR INCUBATION OF THE RESIN FOR 70 HOURS IN DIFFERENT CONCENTRATIONS OF UREA OR GUANIDINIUM HYDROCHLORIDE OR 100 % ETHANOL SIGNIFICANTLY AFFECTS HOST CELL PROTEIN REMOVAL.**

CIP based on thermo-treatment at 90 °C could be a valuable alternative in case commonly used CIP strategies do not fully prevent form bacterial growth. This seems particularly applicable to preparative scale in a stainless steel environment, since lab-scale applications often use temperature sensitive hardware. Further cycling experiments will be employed to shed light on the resin life time after exposure to periodic thermo-treatment.

➨ AUTHORS: **JUDITH VAJDA, ELKE PROHASKA, MAIK RÖHL, EGBERT MÜLLER, TOSOH BIOSCIENCE GMBH**

➨ [1] ECFR — CODE OF FEDERAL REGULATIONS, (N.D.). HTTP://WWW.ECFR.GOV/CGI-BIN/TEXT-IDX?SID=19AA3B822B3B03F66FF441B4D566452F&MC=TRUE&NODE=SE21.2.129_180&RGN=DIV8 (ACCESSED MARCH 10, 2016). [2] GENERAL CHAPTERS: <1231> WATER FOR PHARMACEUTICAL PURPOSES, (N.D.). HTTP://WWW.PHARMACOPEIA.CN/V29240/USP29NF24S0_C1231.HTML (ACCESSED MARCH 10, 2016).
[3] WHO GOOD MANUFACTURING PRACTICES: WATER FOR PHARMACEUTICAL USE, N.D. HTTP://APPS.WHO.INT/PREQUAL/INFO_GENERAL/DOCUMENTS/TRS970/TRS_970_ANNEX2.PDF.

06
GPC
EcoSEC HT

HIGH TEMPERATURE GEL PERMEATION CHROMATOGRAPHY

THE COMPACT HIGH TEMPERATURE SYSTEM EcoSEC HT FOR GEL PERMEATION CHROMATOGRAPHY (GPC) ANALYSIS, THE LATEST ADDITION TO TOSOH'S POLYMER ANALYSIS PLATFORM, IS NOW INSTALLED AND READY FOR MEASUREMENTS IN OUR LAB IN GRIESHEIM. THIS ALL IN ONE GPC/SEC SYSTEM FOR THE ANALYSIS OF ENGINEERING AND COMMODITY PLASTICS, PROVIDES STABLE THERMOSTATIZATION UP TO 220 °C.

Engineering and commodity plastics such as polyamides, nylon, polyphenylene sulfides, polypropylene, and polyethylene excel other materialsby their mechanical strength and their resistance to chemical and physical degradation. However, these advantages turn into hurdles when it comes to their characterization. They are semi-crystalline and need elevated temperatures or special solvents for complete dissolution. This requires specialized instruments and columns to ensure that the sample remains in solution and avoids re-crystallization.

The first step in high temperature GPC 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 optional sample processing unit DF-8321 can process up to 24 samples by moving the sample carefully and applying a temperature program ranging from 40 to 220 °C until full dissolution has occurred. Samples can be filtered automatically to avoid clogging of the GPC column.

EcoSEC HT has a unique dual flow design which includes the use of two pumps under stable temperature atmosphere. The sample pump delivers solvent from the reservoir through the following system components in sequence: autosampler, analytical column and sample side of refractive index (RI) detector cell. At the reference side the solvent flows via the reference pump through a reference column (used to ensure equal pressure conditions) and the reference side of the RI detector. This results in an extremely stable baseline shown in Figure 1 for three different mobile phases.

The RI detector and the temperature controlled pumps of the EcoSEC HT system deliver precise flow rates at all temperatures, even when changes in environmental conditions occur, thus producing reproducible results sample after sample, day after day. For optimum performance the GPC column should be carefully selected to 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 intraday and day to day reproducibility of EcoSEC HT GPC analysis with RI detection at 140°C are shown in Figure 2.

With the installation of the high temperature instrument in our large and modern application lab in Griesheim, we now have two instruments, an ambient system up to 60 °C and an HT system up to 220 °C available to demonstrate the power of EcoSEC for polymer characterization. Interested scientists and researchers are kindly invited to have a look at the systems and get some polymer samples analyzed.

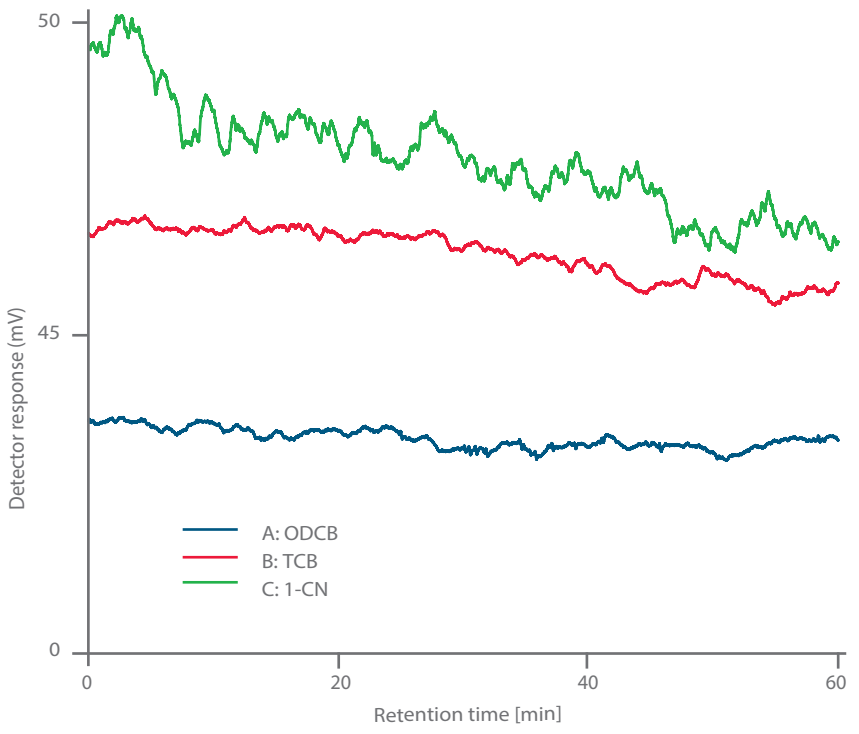


FIGURE 1: BASELINE DRIFT OF THE DUAL FLOW RI DETECTOR OF THE EcoSEC HT GPC SYSTEM FOR ODCB (A, 145 °C), TCB (B, 145 °C), AND 1-CN (C, 220°C) AT 1 ML /MIN WITH 2 X TSKgel GMH$_{HR}$-H (S) HT2

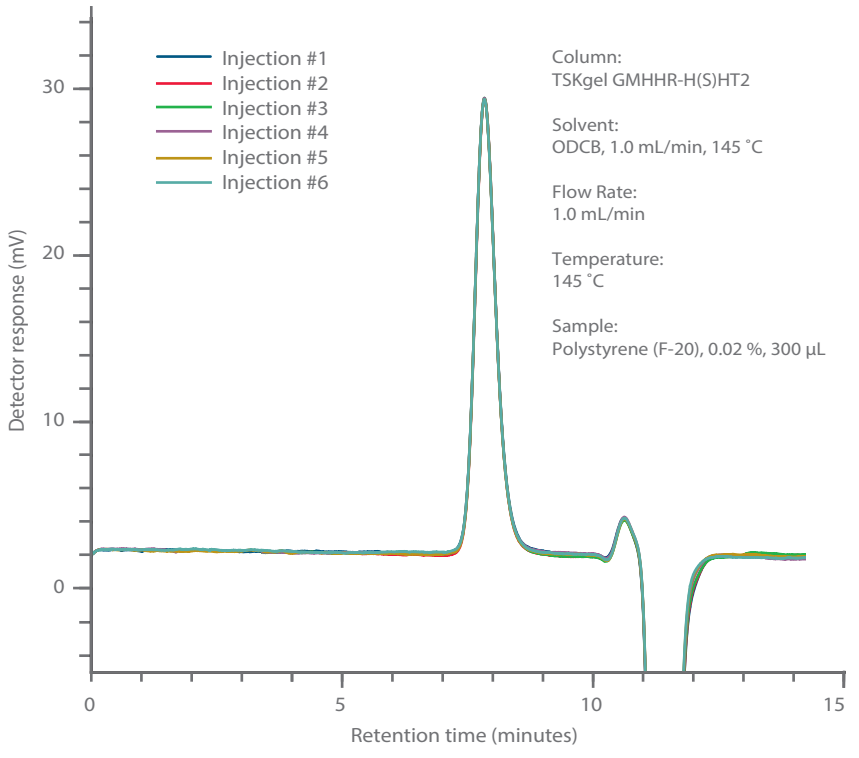


FIGURE 2: GPC ELUTION PROFILE OF INTRADAY REPRODUCIBILITY OF THE EcoSEC HT GPC SYSTEM

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

07 TSKgel IN THE LITERATURE

TO LIKE OR DISLIKE WATER THAT IS THE QUESTION

AMONG THE MANY SCIENTIFIC ARTICLES PUBLISHED ABOUT RESEARCH PROJECTS INVOLVING TSKgel HPLC OR UHPLC COLUMNS WE PICKED TWO VERY RECENT EXAMPLES. CHROMATOGRAPHIC MODES APPLIED WERE HYDROPHOBIC INTERACTION (HIC) AND HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC).

Michal Douša et al. [1] published an article in the Journal of Separation Science on quantification of structurally related aliphatic amino alcohols in l-valinol. They used the popular TSKgel Amide-80 HILIC phase for separation. HILIC separation was combined with postcolumn derivatization and fluorescence detection. L-Valinol is used as intermediate product for production of elvitegravir, an integrase inhibitor used to treat HIV infection. The amino alcohols in l-valinol were effectively separated and quantified with the described method. The influence of the mobile phase (salt type, buffer concentration, and pH) on retention was studied. The TSKgel Amide column (150 × 4.6 mm, 3 µm) used in this study provided well-separated symmetric peaks of analytes with a mobile phase consisting of 10 mM acetate buffer pH 4.0 and acetonitrile (20:80, v/v). After postcolumn derivatization with o-phtaldialdehyde/2-mercaptoethanol fluorescence detection was performed using at an excitation wavelength of 345 and an emission wavelength of 450 nm. After validation, the method was successfully applied to the analysis of commercial samples of l-valinol.

The group of Szabolcs Fekete evaluated hydrophobic interaction chromatography for the separation of monoclonal antibodies and antibody-drug-conjugate species and published two articles on their findings in the Journal of Pharmaceutical and Biomedical Analysis: Part 1 focusing on the optimization of the mobile phase and part 2 on the optimization of the phase system.

Part one published by Marta Rodriguez-Aller et al. [2] provides recommendations for method development in HIC using monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) as model drug candidates. The goal of part 2 published by Alessandra Cusumano et al. [3] was to evaluate the performance of commercially available HIC columns and to develop a fast and automated optimization procedure for the analytical characterization of protein biopharmaceuticals. For this purpose, various therapeutic mAbs (denosumab, palivizumab, pertuzumab, rituximab and bevacizumab) and a cysteine linked ADC (brentuximab-vedotin) were selected as model substances. Several HIC column chemistries (butyl, ether and alkylamide) from different vendors were evaluated in four different buffer systems (sodium acetate, sodium chloride, ammonium acetate and ammonium sulfate). TSKgel Butyl NPR was among the evaluated columns and found to be one of the most versatile ones in terms of hydrophobicity, peak capacity and achievable selectivity. As salt types, ammonium sulfate and sodium acetate were found to be particularly well suited for the analytical characterization of mAbs and ADCs.

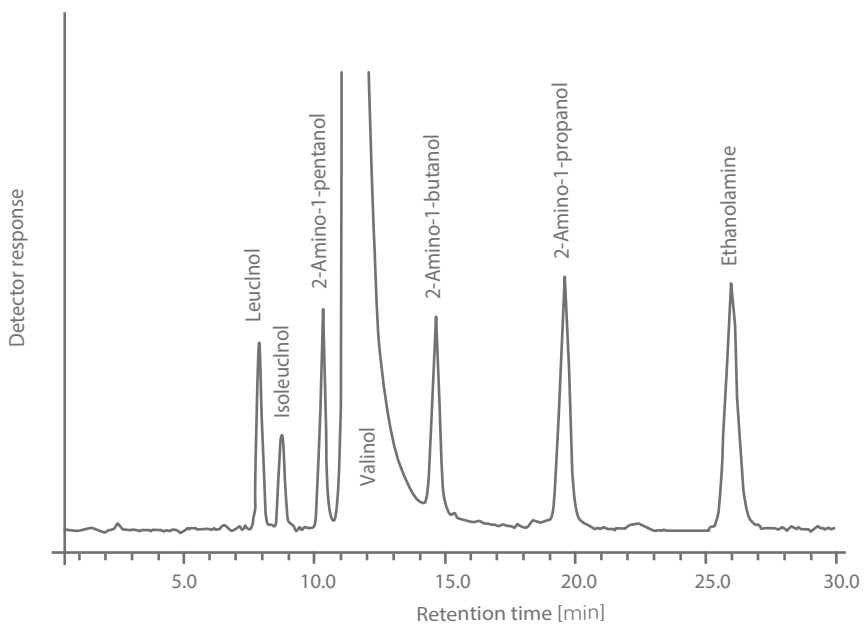
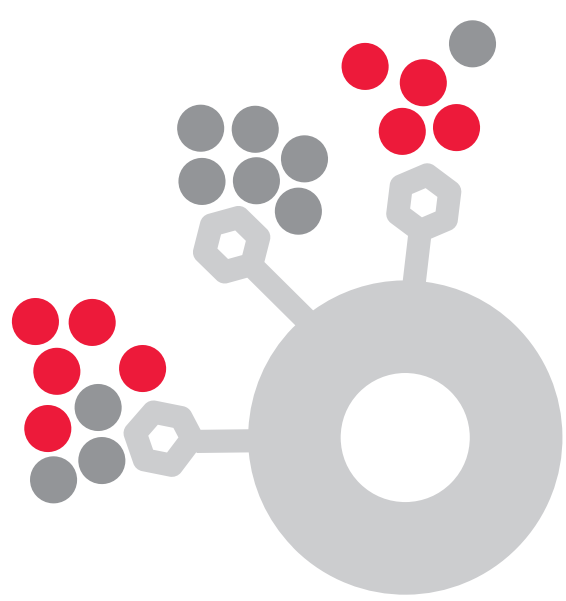


FIGURE 1: **SEPARATION OF AMINO ALCOHOLS ON TSKgel AMIDE HILIC COLUMN AS SHOWN IN REFERENCE 1**



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[2] RODRIGUEZ-ALLER, M., GUILLARME, D., BECK, A., AND FEKETE, S. (2016) J PHARM BIOMED ANAL. 118:393-403; DOI: 10.1016/J.JPBA.2016.11.011

[3] CUSUMANO, A., GUILLARME, D., BECK, A., AND FEKETE S. (2016) J PHARM BIOMED ANAL. 121:161-73; DOI: 10.1016/J.JPBA.2016.01.037

08
WHAT'S HAPPENING
WORKSHOPS

BIOSEPARATION FORUM SERIES & TRAINING COURSES

IN 2016 THE CALENDAR OF TOSOH WORKSHOPS AND TRAINING COURSES IS ALREADY TIGHTLY PACKED WITH EXCITING EVENTS. WE OFFER SEVERAL TRAINING COURSES, SOME OF THEM FOR THE FIRST TIME IN OUR NEW OFFICE IN GRIESHEIM, NEAR FRANKFURT. IN ADDITION TO THE 2- OR 3-DAY COURSES WE WILL BE CONTINUING OUR SERIES OF BIOSEPARATION FORUM EVENTS.

In 2016, we will be offering an international training course on Chromatography in Process Development and Production in our new lab facilities in Griesheim. The course will provide a comprehensive background to chromatographic purification in bioprocessing. Attendees will gather experience in how process method development translates into a productive and cost effective manufacturing process. The program of this 2-day course offers a balance of presentations and practical hands-on experience under the guidance of qualified tutors.

Our renowned basic courses in German language will be held in September at the Technical University Stuttgart. Besides the hands-on training courses we will be hosting our 'Forum Prozesschromatographie' in Griesheim in November. This event in German language consists of a two-day program of presentations of experts out of the field, from other downstream processing suppliers, and from Tosoh.

The event series BioSeparation Forum Chromatography, one-day-seminars on bioseparation, will be continued in June with an event in Ghent, Belgium. This series started in 2012 and provides the latest technical and scientific information about downstream processing. Today, we look back on nine events with more than 300 attendees. The seminars with presentations from Tosoh Bioscience, other suppliers, and from users are free of charge.

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

MEET TOSOH AT TRADESHOWS AND CONFERENCES

UPCOMING EVENTS

MAY 10 - 13	2016	ANALYTICA 2016 | MUNICH [GERMANY]
JUNE 28	2016	PRAXISTAG HPLC | WÜRZBURG [GERMANY]
JUNE 29	2016	BIOSEPARATION FORUM | GHENT [BELGIUM]
SEP. 20 - 23	2016	ILMAC 2016 | BASEL [SWITZERLAND]

TRAININGS | WORKSHOPS

JUNE 15 - 16	2016	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION COURSE IN ENGLISH | GRIESHEIM | [GERMANY]
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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