TOSOH
THE CUSTOMER
MAGAZINE

NO. #01 2010

INSIGHTS / INNOVATIONS / INSPIRATIONS

TOSOH THE ART OF SEPARATION

TOSOH BIOSCIENCE

02 EDITORIAL
DEAR READER

Dear reader, welcome to our first issue of the Tosoh Bioscience customer magazine in 2010. For many suppliers of analysis technology, laboratory technology, and biotechnology the year 2009 has been challenging but the business climate is supposed to be further improving in 2010. The 22nd analytica being the world's leading trade fair for laboratory technology will be the first large event in analytics and biotechnology in Europe. As such it will be regarded as an indicator for the industry's mood in 2010. From March 23 to 26, analytica 2010 and the analytica conference will offer visitors the opportunity to gather information on the latest products and scientific trends and developments.

This issue of our customer magazine will give you an in-depth overview of our latest developments in separation products, completed by practical applications and news out of the field of liquid chromatography. We are looking forward to meeting you at analytica in Munich or at other conferences and exhibitions, such as Bioprocess International in Vienna or ILMAC in Basel.

If you cannot join analytica 2010 but wish to learn more about our products or search our application database, we invite you to discover our Website: www.tosohbioscience.com.

Enjoy reading and stay informed

Regina Roemling / Marketing Manager
Tosoh Bioscience GmbH

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IMPRESSUM

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03
WHAT'S NEW
NEW PRODUCTS

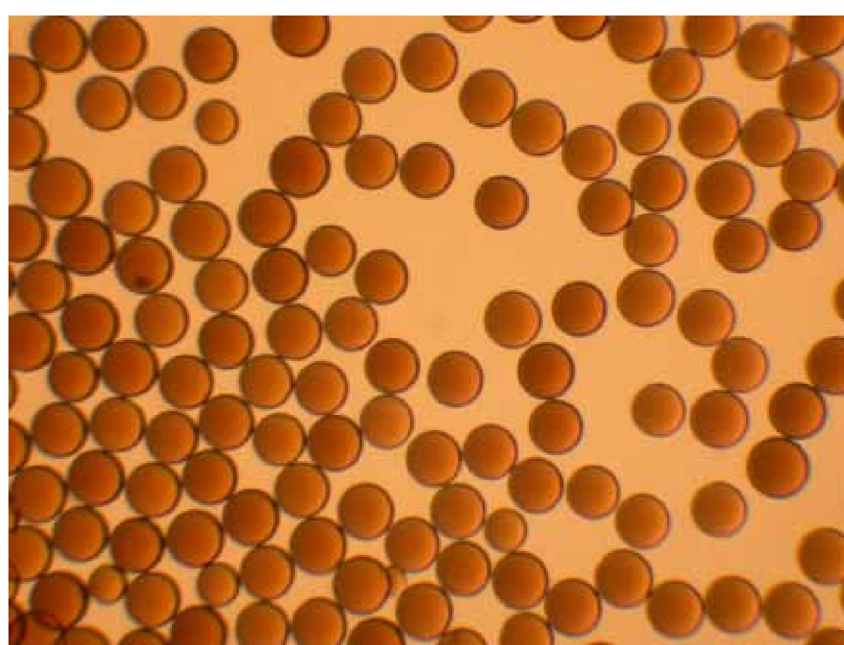
AT ANALYTICA 2010 TOSOH BIOSCIENCE IS SHOWCASING NEW, INNOVATIVE HPLC COLUMNS AND PROCESS RESINS. THESE PRODUCTS EXPAND THE TSK-GEL AND TOYOPEARL PRODUCT RANGE FOR ION EXCHANGE CHROMATOGRAPHY (IEC) AND HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC) SOLUTIONS FOR THE SEPARATION OF BIOMOLECULES.

TOYOPEARL & TSK-GEL PROCESS RESINS: Recently Tosoh Bioscience introduced the Toyopearl GigaCap® series of high capacity ion exchange resins designed for high-throughput purification of biopolymers comprising of GigaCap S-650M and CM-650M cation exchangers and the GigaCap Q-650M anion exchange resin. Due to their exceptional high dynamic binding capacities and excellent pressure/flow characteristics these resins have been highly accepted in the market.
To further expand the range of high capacity ion exchange resins Tosoh Bioscience developed two new resins each targeted to dedicated applications: The new Toyopearl Q-600C AR and the TSKgel SP-3PW. The 'AR' in Toyopearl Q-600C AR stands for 'alkaline resistance' in order to highlight the strength of this resin. It combines a high binding capacity with an enhanced stability towards high pH. This allows harsh CIP and sanitization procedures to be applied without risking a drastic loss in performance. In addition Toyopearl Q-600C AR shows a very narrow particle size distribution, which is favorable for achieving a good packing and high column performance. TSKgel SP-3PW (30) is another new development based on the well established TSKgel SP-5PW resin. TSK-GEL resins are based on a highly cross-linked polymethacrylate particle, which has a higher pressure tolerance than the Toyopearl particle. TSKgel particles are available in 20 µm and 30 µm particle size and therefore are especially suited for polishing steps. TSKgel SP-3PW, having a smaller pore size than the corresponding TSKgel SP-5PW material, was developed to provide high dynamic binding capacities (DBC) for peptides and small proteins. A typical DBC of about 50 g/L for insulin makes this resin attractive for all peptide purification tasks that involve a cation exchange step.

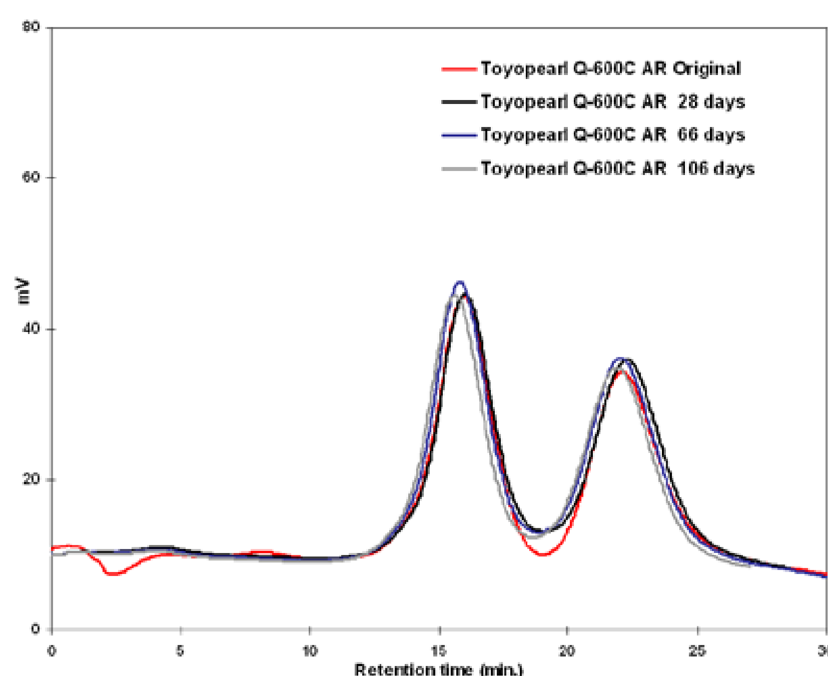
TSK-GEL IEC COLUMNS: Latest surface chemistry, initially developed to create high capacity IEC process resins, was also applied when designing the TSK-GEL STAT IEC stationary phases for HPLC. Since their introduction in 2008 they became a well accepted alternative to conventional or monolithic ion exchange columns for a broad variety of applications such as separation of nucleic acids or quality control of protein biotherapeutics. The TSK-GEL STAT series is based on mono-disperse nonporous particles of which the surface consists of an open access network of multi-layered ion exchange groups. It comprises of strong anion (quaternary ammonium) and weak and strong cation exchange columns (carboxy & sulfo). Dimensions and particle sizes are optimized either for highest throughput or for highest efficiency.

TSK-GEL HILIC COLUMNS: Hydrophilic interaction liquid chromatography (HILIC) is the method of choice for the separation of very polar compounds. TSKgel Amide-80 is the industrial standard for glycoprofiling by HILIC or HILIC-MS. Now a new, robust amino stationary phase expands the selectivity range of TSK-GEL HILIC solutions. The TSKgel NH2-100 column is well suited for all separations requiring amino functional groups. In contrast to many other amino phases it offers expanded stability under HILIC conditions because the NH2-100 phase is treated with a proprietary endcapping procedure. Amino groups are introduced step-wise after endcapping. Due to their high ligand density and large surface TSKgel NH2-100V 3µm columns show high retention for very polar compounds.

MICROSCOPIC IMAGE OF TOYOPEARL Q-600C AR



RT STABILITY OF TOYOPEARL Q-600C AR AFTER EXPOSURE TO 1 N NAOH



04 HPLC APPLICATION

CHARACTERIZING BIOTHERAPEUTICS WITH TSK-GEL HPLC COLUMNS

THE DEVELOPMENT OF NEW BIOPHARMACEUTICALS AND BIOSIMILARS IS A FOCUS OF TODAY'S PHARMACEUTICAL R&D. CURRENT BIOTHERAPEUTICS INCLUDE PEPTIDES SUCH AS INSULIN AND PROTEINS SUCH AS BLOOD FACTORS, RECOMBINANT PROTEINS AND MONOCLONAL ANTIBODIES. THE THOROUGH CHARACTERIZATION OF THERAPEUTIC BIOMOLECULES WITH THE HELP OF A VARIETY OF TECHNIQUES IS A KEY TASK FOR SUBMITTING DATA FOR REGULATORY APPROVALS OF NEW DRUGS. HPLC IS NOT ONLY THE WORKHORSE OF THE PHARMA LAB WHEN ANALYZING SMALL MOLECULE DRUGS BUT IS ALSO POPULAR WHEN ANALYZING LARGE BIOMOLECULES.

The typical chromatographic modes for separation of proteins in native form like size exclusion chromatography (SEC) and ion exchange chromatography (IEC) are routinely used for the characterization of biopolymers. In addition reversed phase (RPC) and hydrophilic interaction liquid chromatography (HILIC) are applied to characterize protein moieties such as small peptides or oligosaccharide chains after enzymatic cleavage. Detection is usually performed by UV, fluorescence or light scattering. Additional structural information can be obtained by applying mass spectrometric detection. TSK-GEL HPLC columns are routinely used in various analytical methods in the biopharmaceutical industry.

Determination of protein aggregates by SEC with TSK-GEL SW columns
Protein aggregation is a common issue encountered during expression, purification and formulation of protein biotherapeutics. It needs to be characterized and controlled during the development of protein pharmaceuticals such as monoclonal antibodies. Even small amounts of aggregates can alter the MAb's function as an effective therapeutic. Aggregation analysis of therapeutic proteins using SEC is almost always required for regulatory approval. SEC on silica based HPLC columns is the common method for separating and quantifying soluble protein aggregates. TSKgel G3000SWXL columns are the industry standard for quality control of monoclonal antibodies.

TSK-GEL SW series columns are based on highly porous silica particles. The surface has been shielded from interacting with proteins by derivatization. When the sample is limited or the components of interest are present at very low concentration, TSKgel SuperSW3000 columns can be applied.

The internal diameter of these columns has been reduced from 7.8 mm to 4.6 mm and the particle size from 5 µm to 4 µm in order to provide higher sensitivity in sample-limited cases. It is important to employ an HPLC system that is optimized with regards to extra-column band broadening to take full advantage of the high column efficiency that can be obtained on TSK-GEL SuperSW columns.

Figure 1 compares the analysis of MAb aggregates on both column types. Separations were performed on an optimized HPLC system equipped with a micro flow cell. Due to the different inner diameters of the columns the flow rates were different in order to apply the same linear flow, resulting in a flow rate of 1 mL/min for the 7.8 mm ID G3000SWXL column and 0.35 mL/min for the 4.6 mm ID SuperSW3000 column. Figure 1 shows that resolution of monomer and aggregate peaks is higher on the SuperSW column, as is the detection sensitivity.

Analysis of charge variants and PEGylated proteins by IEC
Charge isoforms of proteins result from deamidation of asparagine or glutamine residues or from incomplete removal of C-terminal lysine residues. Besides isoelectric focusing cation exchange chromatography is the method of choice to analyze charge heterogeneity of proteins. Newly developed ion exchange columns can help to increase throughput in QC of biopharmaceuticals. TSK-GEL STAT ion exchange columns are non-porous polymer columns with a high surface density of functional groups: quaternary ammonium for anion exchange (Q- and DNA-STAT), carboxymethyl (CM-STAT) and sulfopropyl (SP-STAT) for cation exchange. Particle sizes and dimensions of these IEC columns are optimized either for highest throughput or for highest efficiency.

Figure 1: Determination of MAb aggregates by SEC. TSKgel G3000SWXL column (5 µm, 7.8 mm ID x 30 cm L); flow rate 1 mL/min; TSKgel Super SW3000 column (4 µm, 4.6 mm ID x 30 cm L); flow rate 0.35 mL/min; mobile phase: 0.1 M phosphate pH 6.8, injection volume 5 µl, detection: UV@280 nm (micro flow cell)

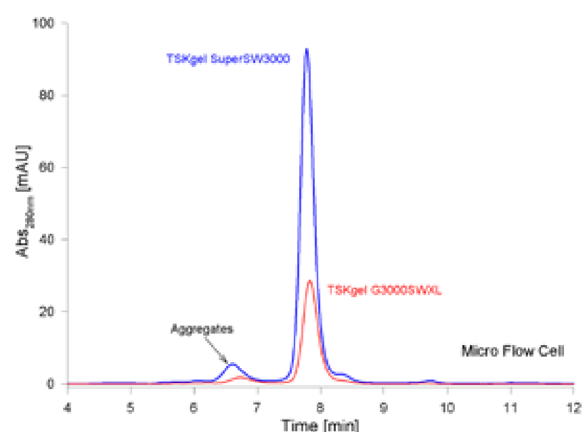
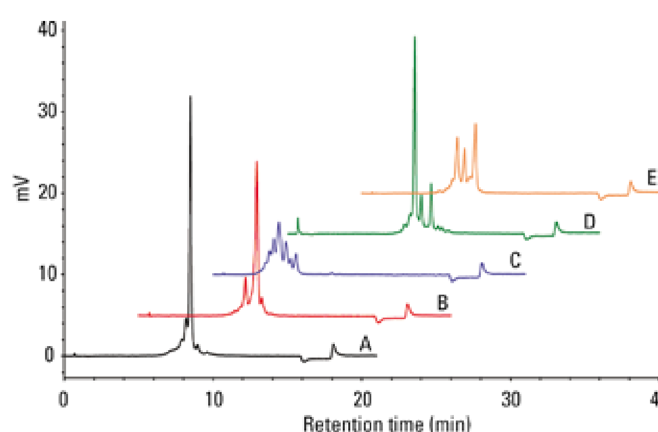


Figure 2: Separation of MAb charge variants by IEC TSKgel CM-STAT column (7 µm, 4.6 mm ID. x 10 cm L); mobile phase: A: 20 mM MES (pH 6.0), B: 20 mM MES + 0.5 M NaCl (pH 6.0); gradient 10% B to 15 % B in 15 min; flow rate: 1 mL/min; detection: UV@280 nm, injection volume 20 µL

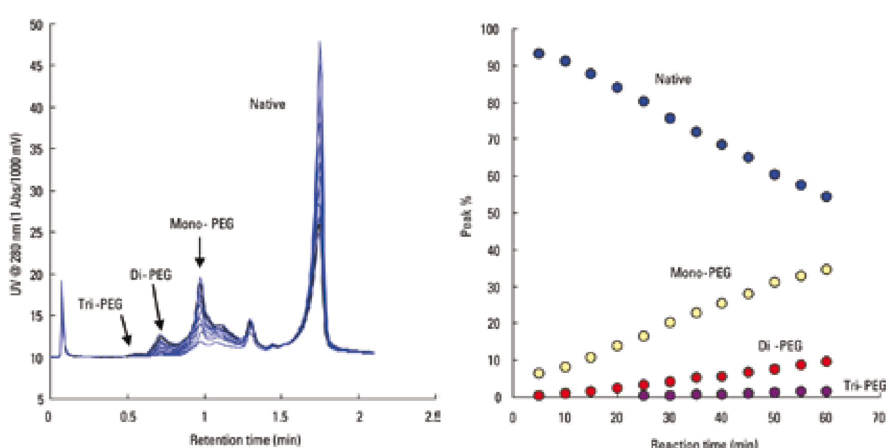


A weak cation exchange (WCX) column can be applied to separate charge variants of several monoclonal antibodies (Figure 2). The typical analysis time on conventional 25 cm long WCX columns of about forty minutes can be significantly reduced when separation was performed on a 10 cm TSKgel CM-STAT column, filled with 7 µm particles. The analysis profiles for five antibodies show that high resolution analysis can be obtained in about 20 minutes analysis time.

PEGylation, the process by which polyethylene glycol (PEG) chains are attached to protein drugs is a common practice to improve pharmacokinetics of a biopharmaceutical. Lysine residues of the protein typically serve as PEGylation sides. The degree of PEGylation can be monitored by analyzing the increase in molecular weight by SEC, as every PEG group remarkably increases the apparent size of the protein. As every PEG residue also shields parts of the protein and consequently alters the overall surface charge, ion exchange separation can be also used. SEC separation requires a certain analysis time and is not well suited for high throughput applications. IEC, however, can be accelerated as shown above. In addition IEC has the ability to resolve isoforms differing in the position of PEGylated lysine residues.
Figure 3 shows the monitoring of a PEGylation reaction of ß-Lactoglobulin in intervals of 5 minutes on a high-throughput TSKgel SP-STAT strong cation exchange (SCX) column. A fast gradient of sodium chloride in sodium acetate buffer at high flow rate of 2 mL/min was applied to achieve fast separation.

Characterization of protein glycosylation by HILIC with TSKgel Amide-80
Another important QC parameter of MAb characterization is the analysis of glycosylation as it may influence immunogenicity, pharmacokinetic and pharmacodynamic properties. Several complementary analytical techniques are routinely used to characterize, identify, and quantify oligosaccharides isolated from glycoproteins. The standard method for QC analysis of glycans is the HILIC separation of AB-labeled glycans. Tagging the glycans with a fluorescent label such as 2-aminobenzamide (2-AB) allows the sugars to be detected at femtomole levels. HILIC can separate structures with the same composition (isobaric glycoforms) on the basis of both sequence and linkage.

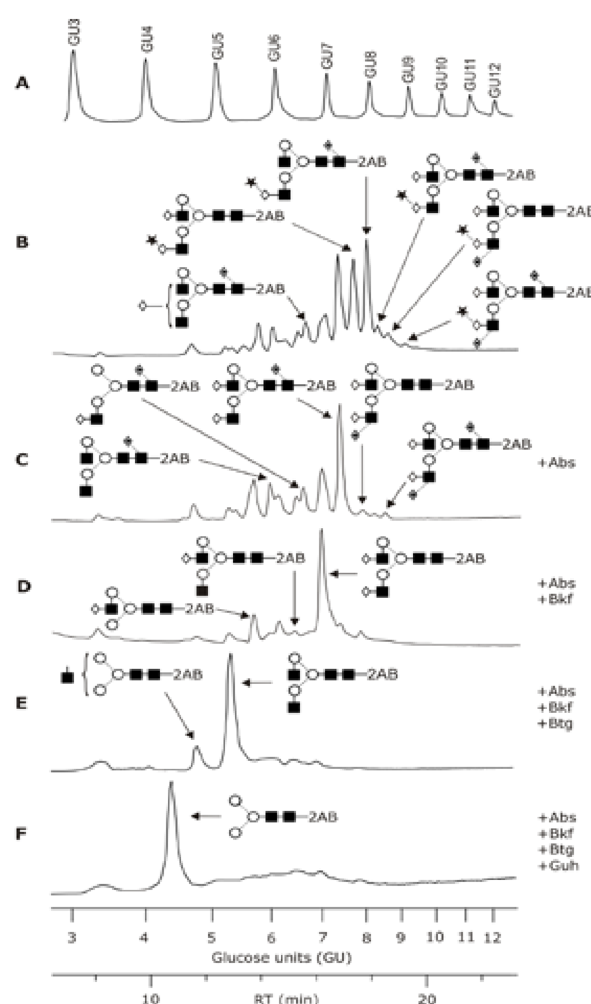
Figure 3: High frequency monitoring of a PEGylation reaction TSKgel SP-STAT column (10 µm, 3.0 mm ID x 3.5 cm L); mobile phase: A: 20 mM sodium acetate (pH 5.0), B: 1.0 M NaCl in buffer A (pH 5.0), 2 min gradient 0 - 100% B; flow rate: 2 mL/min; detection: UV @ 280 nm



The chemistry of amide bonded HILIC phases is ideally suited for the separation of charged and neutral fractions of glycan pools in one run. The retention of fluorescence labeled polysaccharides on TSKgel Amide-80 enables the identification of glycan structures by comparison to a labeled dextran ladder. The dextran ladder is used to normalize retention times in order to calculate the number of glucose units (GU values) of the separated glycans. The GU values obtained after separation of sequential exoglygosidase digests can be used to predict the glycan structure by database query (Glycobase, autoGU). Figure 4 shows HILIC separations of 2-AB labeled N-glycans released from a recombinant ZP domain construct of murine transforming growth factor beta type 3 receptor (TGFR-3).

Summary TSK-GEL HPLC columns are applied in a broad range of HPLC applications used in development, production, validation and release of protein therapeutics. Some of the applications mentioned above are mandatory when submitting a new biopharmaceutical to approval at the regulatory agencies. The introduction of the first so-called biosimilars in Europe further increased the need for highly efficient analysis methods. Tosoh Bioscience is continuously developing new stationary phases, thus supporting biochemists in increasing lab productivity and establishing high throughput HPLC analysis for biopolymers.

Figure 4: HILIC chromatograms of 2-AB labeled glycans Dextran ladder (A), PNGaseF digest (B), sequential exoglycosidase digests (C-F). Used exoglycosidases: Sialidase A (Abs), α-Fucosidase (Bkf), β-Galactosidase (Btg), β-N-Acetylhexoamidase (Guh) TSKgel Amide-80 column (3 µm, 2 mm ID x 15 cm L); mobile phase: A: 50 mM ammonium formate (pH 4.3), B: acetonitrile , 35 min. gradient 75 - 35 % B; flow rate: 0.22 mL/min; temperature:50 °C; detection: fluorescence; excitation @ 360 nm, emission @ 425 nm; injection volume: 2 µL (approximately 300 fmol for GU3) (courtesy of K. Darsow, S. Bartel & H. Lange, University of Erlangen-Nuremberg)



06 TOYOPEARL & TSK-GEL IN THE LITERATURE

DEVELOPMENT OF A HIGH-CAPACITY MAB CAPTURE STEP

DUE TO CONFIDENTIALITY IN BIOPHARMACEUTICAL INDUSTRY IT IS VERY RARE FOR CUSTOMERS TO PUBLISH THEIR CURRENT EVALUATION AND PROCESS PERFORMANCE DATA ON OUR TOYOPEARL® RESINS. THEREFORE IT IS A PLEASURE TO CITE HERE AN ARTICLE FROM BLANCA LAIN ET AL.1 (PERCIVIA LLC) WHICH WAS PRESENTED IN THE MAY 2009 EDITION OF BIOPROCESS INTERNATIONAL. IT ADDRESSES THE CURRENT INDUSTRY CHALLENGE OF IMPROVING THE DOWNSTREAM THROUGHPUT OF TODAY'S HIGHER TITER MAB FEEDSTOCKS WHILE TRYING TO REDUCE PROCESS COSTS.

Protein A affinity chromatography is traditionally used as capture step for monoclonal antibodies (MAbs). Due to its high specificity the drawbacks such as high price, large column sizes due to limited dynamic binding capacities and removal of leaching protein A were accepted. But as antibody expression levels have significantly increased to more than 10 g/L in fed-batch cultures the biopharmaceutical industry seeks alternative downstream processing solutions for purification of MAbs. Blanca Lain and coworkers report their results in developing a high-capacity MAb capture step using high-capacity cation exchange chromatography. Using an IgG1 antibody expressed in their proprietary PER.C6® cell line expression system they demonstrate that Toyopearl GigaCap S-650M can capture MAbs from clarified harvests with a binding capacity of > 90 g/L, > 95 product recovery, and purity comparable to that of protein A including > 95% HCP reduction. When compared to CaptoTM S (GE Healthcare) the Toyopearl GigaCap S-650M delivered 33% more DBC for IgG1 (100 mg/mL vs 75 mg/mL), 50% reduction in elution pool volume (~5CV vs > 10CV). The favorable elution kinetics of Toyopearl GigaCap S-650M leading to reduced elution pool volumes were also reported by Al Jackewitz[2].

A loading operating window was evaluated by DOEs with respect to loading buffer pH and conductivity. The effects of load concentration of the MAb and residence times on binding capacity, yield and product purity were analyzed as well (see data below). To evaluate elution conditions a DOE study was performed which investigated pH and the NaCl concentration of the elution buffer.

Lain *et al*. showed that Toyopearl GigaCap S-650M can be operated at linear flow rates up to 900 cm/h (over 1 min residence time) without significant loss of capacity. Together with the good pressure flow characteristics this will directly affect the duration and performance of this capture step. At optimal loading and elution conditions as determined by a series of DOEs the results of this cation exchange step were reported to be comparable to those of an affinity capture step.

EFFECT OF RESIDENCE TIME ON BINDING CAPACITY, YIELD, AND HCP REDUCTION[1]

Residence Time (min)	Total DBC (g MAb/L resin)	Yield (%)	HCP Reduction (%)
2	101	97.5	95
1.4	101	95.2	95
1	99	95.9	95

EFFFECT OF LOAD CONCENTRATION ON THE CEX CAPTURE STEP[1]

MAb load concentration (mg/ml)	Yield (%)	Mass Balance (%)	DBC (g/L)	HCP Reduction (%)
0.5	97	97	90	97
2.6	97	97	91	96
7.7	90	91	79	95

References:

1- Blanca Lain, Marco A. Cacciuttolo & Gregory Zarbis-Papastoitsis. Development of a High-Capacity MAb Capture Step Based on Cation-Exchange Chromatography. BioProcess International 7 (5) 2009: 26-34

2- Al Jackewitz. Reducing Elution Pool Volumes with High Capacity and Improved Mass Transfer Ion-Exchange Resins. BioProcess International 6 (7) 2008: 108-110

07 GPC ECOSEC TOUR

ECOSEC SEMINAR TOUR 2009/2010

TO DEMONSTRATE NEW AND INNOVATIVE SOLUTIONS FOR SUSTAINABLE GPC/SEC - THIS WAS THE AIM OF THE ECOSEC TOUR 2009, WHICH WILL BE CONTINUED IN 2010. A ONE DAY FREE-OF-CHARGE SEMINAR WITH WORKSHOPS HELD BY TOSOH BIOSCIENCE GMBH AND POLYMER STANDARDS SERVICE PSS AT VARIOUS PLACES THROUGHOUT GERMANY AND THE NETHERLANDS IN 2009 PROVIDED LATEST INFORMATION ABOUT THE ECOSEC SEMI-MICRO GPC SYSTEM AND CURRENT CHALLENGES AND APPLICATIONS OF GPC/SEC. MORE THAN 100 PARTICIPANTS JOINED THE SEMINARS IN 2009.

One of the major parts of this seminar is to learn more about different GPC/SEC modes and about semi-micro GPC/SEC, a technique that became available with the EcoSEC system for the first time. The main advantage of semi-micro GPC/SEC is that it allows to save up to 65% eluent with every sample.

From the many features of EcoSEC the revolutionary dual-flow technology of the EcoSEC refractive index (RI) detector attracted a great deal of attention. It is designed in a way that the pure mobile phase is permanently rinsing the reference site of the RI detector cell. Tedious purging of the RI with pure solvent using a purge valve at the start of a batch and after changing the solvent bottle becomes redundant. With EcoSEC extraordinary fast equilibrium and setup times can be achieved. The solvent in the sample site and the reference site of the cell has always the same quality and temperature. This leads to extremely stable baselines allowing a proper integration and processing of smallest peaks. Apart from the dual-flow design the RI convinces also with the small size of its detector cell. The small volume of only 2.5 µl minimizes peak broadening and artefacts. This enables EcoSEC to be used not only for analytical SEC/GPC but also in semi-micro mode, which saves reasonable amounts of solvent. Another reason for baseline stability is the dual thermostatted column compartment, which accommodates up to 8 columns of up to 30 cm length. This oven-in-the-oven concept allows stable operation of the EcoSEC even under the most challenging conditions, such as direct insolation or strong draft.
In 2010 PSS and Tosoh Bioscience GmbH will provide eight additional opportunities to join the international EcoSEC workshop tour with live presentations about modern GPC/SEC solutions:

- March 16, 2010: Basel, Switzerland
- April 20, 2010: Milan, Italy
- May 17, 2010: Paris, France
- October 12, 2010: Lyon, France
- June 16, 2010: Krakow, Poland
- September 08, 2010: Frankfurt, Germany
- October 06, 2010: Copenhagen, Denmark
- October 27, 2010: Barcelona, Spain

For more information and registration visit www.ecosec.eu

CONFERENCE ANNOUNCEMENT

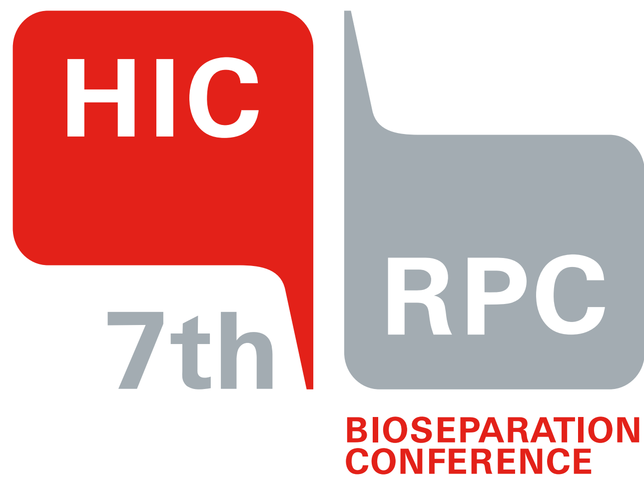
7TH HIC/RPC BIOSEPARATION CONFERENCE 2011

THE HIC/RPC BIOSEPARATION CONFERENCE SERIES, WHICH ALTERNATES BETWEEN EUROPE AND THE US, PROVIDES A UNIQUE FORUM FOR IN-DEPTH DISCUSSIONS ON DOWNSTREAM BIOPROCESSING. THE CONFERENCE IS FOCUSED ON THE BETTER UNDERSTANDING OF THE HYDROPHOBIC NATURE OF BIOLOGICAL TARGETS AND THEIR CHROMATOGRAPHIC ISOLATION AND PURIFICATION BASED ON THIS UNDERSTANDING. TRADITIONALLY THE CONFERENCE SEEKS TO MAINTAIN AN OPTIMAL BALANCE OF FUNDAMENTAL SCIENCE AND INDUSTRIAL ADVANCES AND APPLICATIONS.

The 7th HIC/RPC Bioseparation Conference will be held from March 21-24, 2011 at the Hotel Palacio in Estoril near Lisbon, Portugal. This conference follows a successful 6th HIC/RPC meeting at the Silverado Resort in Napa Valley, CA. Tosoh Bioscience is the sole sponsor of the conference and provides support for logistics and organisation for the scientific committee. The scientific committee of the 7th HIC/RPC conference will be headed by Professor Dr. Alois Jungbauer, University of Natural Resources and Applied Life Science (BOKU), Vienna, Austria. The first announcements and call for abstracts will be starting in May 2010.

TO LEARN MORE ABOUT THE HIC/RPC BIOSEPARATION CONFERENCE SERIES PLEASE VISIT THE CONFERENCE WEBSITE WWW.HIC-RPC.ORG

BIOSEPARATION CONFERENCE



HIC/RPC CONFERENCE VENUE

NEWS & EVENTS | MEET TOSOH BIOSCIENCE

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

UPCOMING EVENTS

MARCH	23 - 26	2010	ANALYTICA 2010, MUNICH [GERMANY]
MAY	19 - 20	2010	EUROPEAN BIOPROCESS INTERNATIONAL 2010, VIENNA [AUSTRIA]
JUNE	21 - 23	2010	ARBEITSTAGUNG MARTINSRIED 2010, MARTINSRIED [GERMANY]
SEP	05 - 08	2010	ISPPP 2010, BOLOGNA [ITALY]
SEP	21 - 24	2010	ILMAC 2010, BASEL [SWITZERLAND]
NOV	08 - 09	2010	NOVIA HPLC-TAGE 2010, FRANKFURT [GERMANY]

TRAININGS | WORKSHOPS

APRIL	13 - 15	2010	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION | BASIC COURSE | STUTTGART [GERMANY]
APRIL	20 - 22	2010	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION | BASIC COURSE | STUTTGART [GERMANY]
APRIL	27 - 29	2010	CHROMATOGRAPHY IN PROCESS DEVELOPMENT & PRODUCTION | BASIC COURSE | STUTTGART [GERMANY]

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