TOSOH

TOSOH THE CUSTOMER MAGAZINE

NO. #02 2020

VIRUS/VECTOR/VIRTUAL

TOSOH BIOSCIENCE

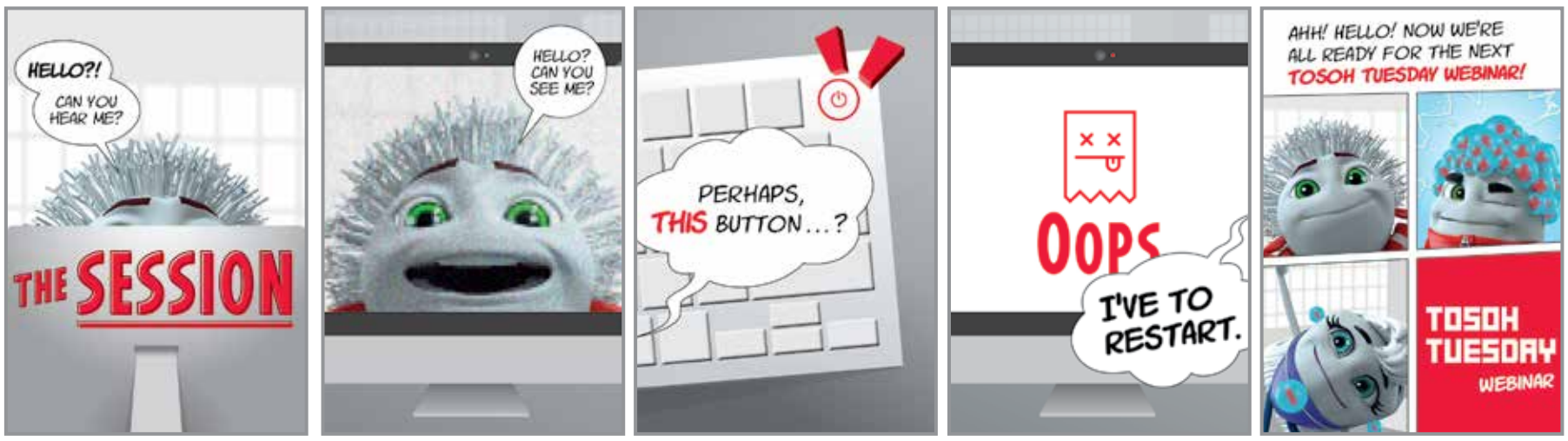
02
EDITORIAL
DEAR READER

Welcome to the second issue of the Tosoh Bioscience customer magazine in 2020. When we turned the first issue of the customer magazine into a COVID-19 issue we were hoping that it would be a unique case. Now we are 6 months ahead and still / again in a Corona way of life.
Besides the many restrictions this pandemic caused we noticed that on the other hand it accelerated many developments such as working from home offices, digitalization, virtual tradeshows and conferences. The circumstances forced us to change procedures and today we all are familiar with virtual events and meetings. We successfully presented our renowned BioSeparation Forum in virtual format and will continue to connect with you on all available channels. Nevertheless, we hope to be able to meet you again in person in 2021 and wish you and your family a healthy and happy year 2021.

STAY HEALTHY AND ENJOY READING

REGINA ROEMLING | SENIOR MARKETING MANAGER
TOSOH BIOSCIENCE GMBH

SUPER-T, HIS FRIENDS AND THE VIRTUAL SPACE



CONTENT

IMPRESSUM

TOSOH BIOSCIENCE GMBH
IM LEUSCHNERPARK 4 | 64347 GRIESHEIM | T: +49 [0] 6155 70437-00 | F: +49 [0] 6155 8357900 | INFO.TBG@TOSOH.COM

J20LP19A

03 VIRUS CORONA

CHROMATOGRAPHIC SOLUTIONS FOR COVID-19 VACCINE DEVELOPMENT

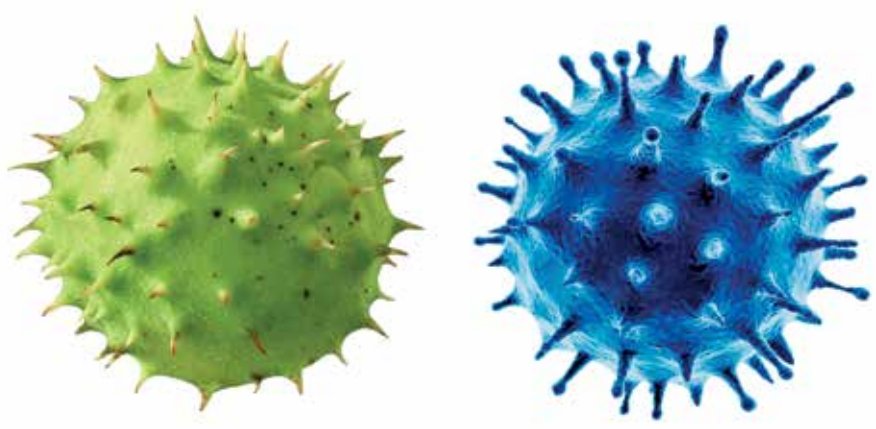
TOSOH BIOSCIENCE HAS BEEN ENABLING ITS BIOPHARMA PARTNERS TO PROVIDE ROBUST DIAGNOSTICS SOLUTIONS AND SAFE AND EFFICIENT THERAPIES FOR LIFE-THREATENING DISEASES FOR DECADES. DURING THE ON-GOING BATTLE AGAINST COVID-19, THE NECESSARY STEPS HAVE BEEN TAKEN TO ENSURE BUSINESS CONTINUITY AND TO SUPPORT THE ON-GOING R&D AND PRODUCTION ACTIVITIES. WE OFFER A VARIETY OF CHROMATOGRAPHY RESINS AND COLUMNS TO SUPPORT RESEARCH AND DEVELOPMENT IN THE FIGHT AGAINST COVID-19.

This article summarizes some scientific papers using either TSKgel columns for analysis of SARS-CoV-2 proteins as well as vectors, viruses and VLPs that could be used as therapies or vaccines or TOYOPEARL resins for purification of these.

Li et al.[1] analyzed the oligomerization of recombinant versions of SARS-CoV-2 spike protein S (S, S1 and RBD) expressed in insect cells using TSKgel G5000PW$_{XL}$ SEC columns. The same column can be applied to quantify and characterize enveloped virus-like particles by SEC-UV and SEC-MALS as demonstrated by Steppert et al.[2]. J. Vajda et al.[3] used a SEC column with larger pore size, TSKgel G6000PW$_{XL}$, for size distribution analysis of influenza virus particles to complement the hemagglutination assay. The universal applicability was demonstrated with three different influenza virus samples.

Plasmid vectors of various sizes can be analyzed by SEC, HIC and anion exchange chromatography. In a frequently cited article J. Urthaler et al. described the use of a TSKgel DNA-NPR anion exchange column to analyze topoisomers of DNA plasmids expressed in *E. coli*.[4] J. Nelson et al. preferred using hydrophobic interaction chromatography on TSKgel Butyl-NPR for plasmid isoform evaluation of the final product of an antibiotic-free production of a herpes simplex virus 2 DNA vaccine in a high yield cGMP process. Enrichment of supercoil plasmid form was accomplished using hydrophobic interaction chromatography (HIC) on TOYOPEARL Butyl-650M. The HIC load conditions allowed the retention of supercoiled plasmid DNA and certain contaminants while open circle plasmid forms and residual host cell DNA passed through the packed resin bed. The elute conditions resulted in the selective elution of supercoiled pDNA[5].

A technology that can be applied for the delivery of siRNA was described by W. Shao et al.[6] . They purified novel polyethyleneimine -coated adeno-associated virus-like particles for siRNA delivery with TOYOPEARL Butyl-650M HIC resins. The delivery of small interfering RNA (siRNA) is limited by its poor stability and limited cell-penetrating properties. To overcome these limitations, they designed an efficient siRNA delivery system using polyethyleneimine-coated virus-like particles derived from adeno-associated virus type 2.



REFERENCES:

1 – Characterization of the SARS-CoV-2 Spike in an Early Prefusion Conformation; T. Li et al.; bioRxiv, 17 Mar 2020; DOI: 10.1101/2020.03.16.994152

2 – Quantification and characterization of virus-like particles by size-exclusion chromatography and nanoparticle tracking analysis; P. Steppert et al.; J Chromatogr A 2017 Mar 3; 1487:89-99. DOI: 10.1016/j.chroma.2016.12.085

3 – Size distribution analysis of influenza virus particles using size exclusion chromatography; J. Vajda et al.; J Chromatogr A 2016 Sep 23;1465:117-25. DOI: 10.1016/j.chroma.2016.08.056

4 – Improved downstream process for the production of plasmid DNA for gene therapy; J. Urthaler et. al.; Acta Biochimica Polonia; Vol. 52 No. 3/2005, 703–711. DOI: 10.18388/abp.2005_3434

5 – Antibiotic-free production of a herpes simplex virus 2 DNA vaccine in a high yield cGMP process; J. Nelson et al.; Human Vaccines & Immunotherapeutics Volume 9, 2013 - Issue 10, 2211-2215 DOI: 10.4161/hv.25048

6 – A novel polyethyleneimine-coated adeno-associated virus-like particle formulation for efficient siRNA delivery in breast cancer therapy: preparation and in vitro analysis; W. Shao; Int J Nanomedicine. 2012; 7: 1575–1586. DOI: 10.2147/IJN.S26891

04
FOCUS
NEW THERAPIES

OLIGONUCLEOTIDES: PERFECTING PURIFICATION

OLIGONUCLEOTIDE-BASED THERAPEUTICS HAVE BEEN INVESTIGATED OVER THE LAST DECADES AND THEIR PROMISE AS A NEW DRUG MODALITY IS NOW BEING REALIZED. THE GROWING INTEREST IN OLIGONUCLEOTIDES IS DRIVEN BY THEIR HIGH POTENTIAL TO BE USED IN THE TREATMENTS OF A VARIETY OF MEDICAL CONDITIONS, THE GROWING NUMBER OF FDA APPROVED OLIGONUCLEOTIDE DRUGS, AND AN INCREASED FOCUS ON PERSONALIZED MEDICINE AND ON THE DEVELOPMENT OF THERAPIES FOR RARE DISEASES.

Two major types of oligonucleotide drugs are currently being developed as therapeutic platforms for the reduction of target gene expression: short interfering RNA (siRNA) and antisense oligonucleotides (ASOs). The potential of aptamer RNAs that modulate protein functions is another popular area of research.

For the companies pursuing oligonucleotide based therapies, the relative complexity of their synthesis can pose purification challenges. Oligonucleotides are short DNA or RNA molecules that are "grown" via the addition of nucleotide groups in a series of solid-phase synthesis cycles – a failure in this process can allow impurities to emerge. Some chemical modifications are necessary to improve their pharmacokinetics and stability. These could result in another source of impurities. Irregularities in the manufacturing process can lead to the formation of the following common impurities:

- Shortmers – oligonucleotides missing one or more nucleotides, or N-1
- Longmers – oligonucleotides that include more than the intended number of nucleotides, or N+1
- Lack of protecting groups (derivatives of existing functional groups that decrease reactivity and increase stability)
- Phosphodiester impurities (PO) in phosphorothioate oligonucleotides

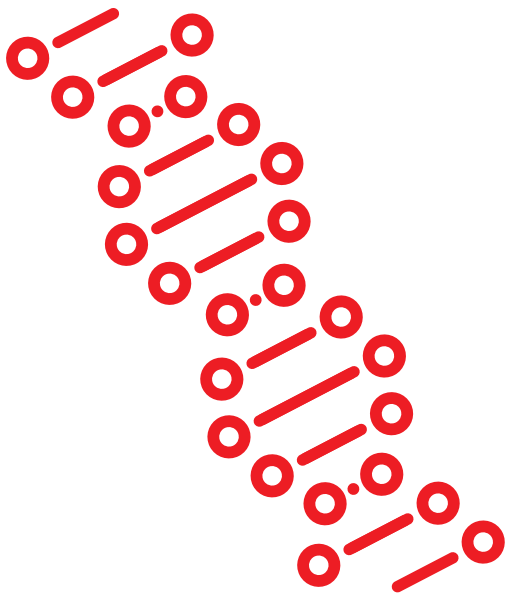
Fortunately, when it comes to removing impurities that occur during oligonucleotide manufacturing, a range of chromatographic techniques ensure that the highest levels of purification can be achieved. Anion exchange (AIEX) chromatography is the optimal solution for addressing variation in strand lengths – shortmers and longmers. Because siRNAs are highly polar by nature, the high performance anion-exchange medium, TSKgel SuperQ-5PW (20), can easily separate unwanted synthetic strands from the desired product. This resin offers both high resolution and high loading capacity, which is essential from a productivity point of view.

A novel, effective and high recovery method for purification of a DMT-on oligonucleotide and the effective removal of a DMT-group from an oligonucleotide in a single purification step can be achieved by using hydrophobic interaction chromatography (HIC).

Dimethoxytrityl (DMT), a 5′ protecting group, is used in the synthesis of oligonucleotides to temporarily mask the characteristic chemistry of a 5′-hydroxy functional group. In many preparations of delicate oligonucleotides, DMT may be left on an oligonucleotide following synthesis to give stability to the molecule during subsequent processing. Purification by HIC can be achieved since the DMT-on group is strongly hydrophobic. On-column cleavage at low pH effectively splits the oligonucleotide from the bound DMT-group and elutes a DMT-off oligonucleotide in high purity and recovery.

Characterization and quality control of oligonucleotides essentially apply the same modes of chromatography as purification. In AIEX chromatography, TSKgel (U)HPLC columns based on non-porous particles are available with the same surface and ligand chemistries as the TOYOPEARL and TSKgel purification media. TSKgel DNA-STAT or DNA-NPR columns offer fast kinetics and high-resolution separation.

Size exclusion chromatography (SEC) can also be applied in combination with light scattering detection. The TSKgel UP-SW2000 UHPLC column can separate oligonucleotides differing by one base in length. And when coupled with our new LenS$_3$ multi-angle light scattering (MALS) detector, a detailed picture of the oligonucleotide purity can be achieved.



SEE ALSO THE MEDICINE MAKER ISSUE OCTOBER 2020: bit.ly/TMM-Oligo

05 FOCUS NEW THERAPIES

PLASMID VECTORS: ANALYSIS OF TOPOISOMERS

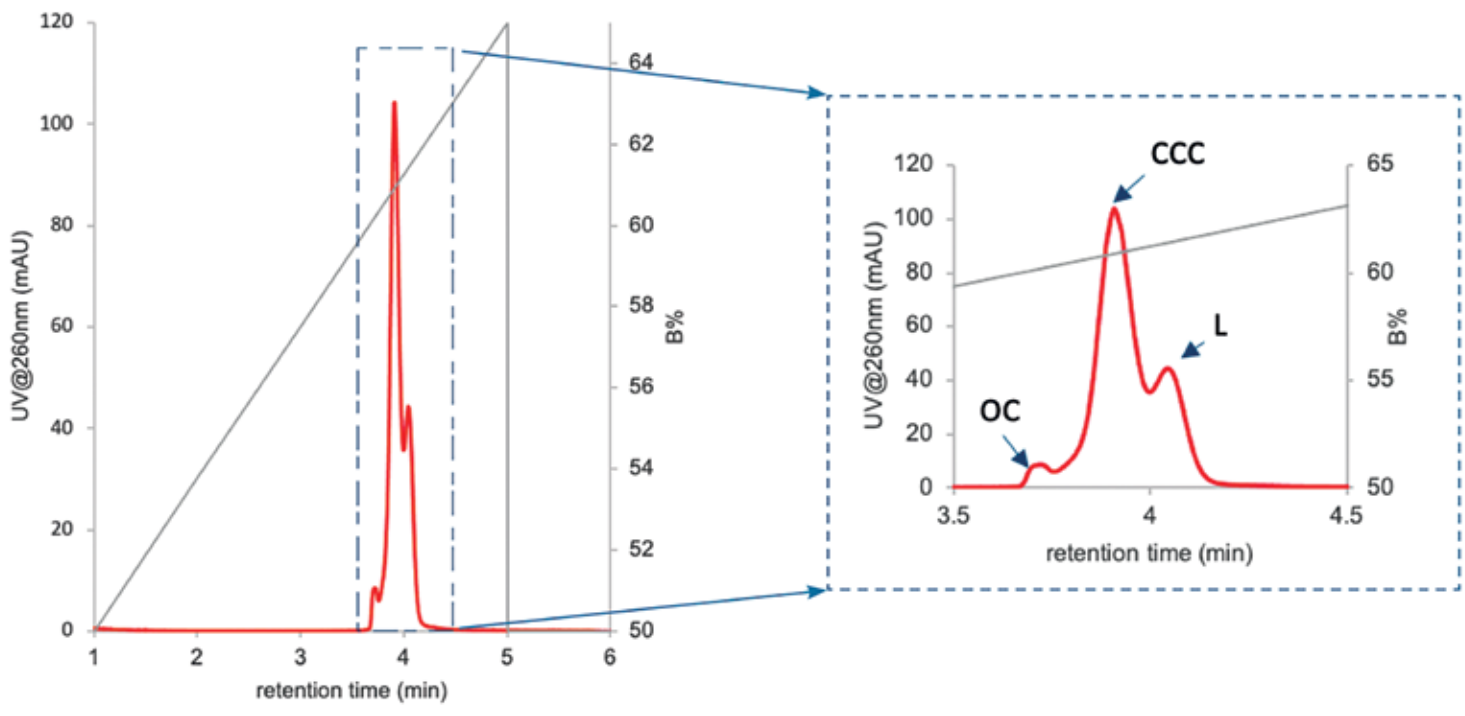
RECOMBINANT PLASMID DNA (pDNA) IS INCREASINGLY USED AS A RAW MATERIAL IN GENE THERAPY AND AS AN ACTIVE INGREDIENT FOR DNA VACCINATION. PHARMACEUTICAL GRADE PLASMID DNA MUST MEET SPECIFICATIONS CONCERNING THE CONTENT OF PLASMID DNA TOPOISOMERS (SUPERCOILED, OPEN CIRCULAR, AND LINEAR). AN ANION EXCHANGE HPLC METHOD WITH HIGH RESOLVING POWER EMPLOYS THE TSKgel DNA-NPR ANION EXCHANGE HPLC COLUMN TO RAPIDLY ANALYZE DNA TOPOISOMERS.

Plasmid DNA that is intended to be used for therapeutic purposes must meet specifications concerning both host-related-impurities as well as homogeneity (i.e. the content of pDNA topoisomers (ccc, oc, lin) and di- or multimeric variants). During large-scale plasmid fermentation, plasmids are maintained predominantly in a supercoiled, covalently closed circular form. During downstream processing some of the plasmids might become nicked and they will be transformed in open-circular and linear forms.

For a fast and successful characterization of pDNA samples Schuchnigg et al.1 developed an HPLC method with a high resolving power based on the TSKgel DNA-NPR anion exchange HPLC column. TSKgel DNA-NPR is packed with 2.5 µm hydrophilic non-porous polymer beads modified with a weak anion exchange group. The non-porous particle offers fast mass transfer, a key to achieve high resolution. The small particle size and the fast mass transfer of non-porous beads can be exploited to speed up the analysis.

The plasmid pBR322 has a length of 4,361 base pairs, which results in a molecular weight of 2.83 x 10^6 Da. In order to classify the topoisomers properly, the linear form was prepared by incubation with the single-cutting restriction Enzyme EcoRI (NEB). pBR322 has a single EcoRI restriction site at position 4359.

The chromatographic analysis of pBR322 on TSKgel DNA-NPR is shown in the figure below. The plasmid can be analyzed within a 5 min linear gradient from 50% to 65% mobile phase B at a flow rate of 1.0 mL/min. The plasmid elutes in three peaks representing the different species, supercoiled, open circular, and linear. The highest peak corresponds to the supercoiled plasmid. Schuchnigg et al. showed that the elution pattern changes with plasmid size. Therefore, it is recommended to confirm the position of the peak representing the linear form. The method is ideally suited to be used at various stages of pDNA R&D and manufacturing for pharmaceutical purposes and can be used in both, HPLC and UHPLC systems.



ANION EXCHANGE ANALYSIS OF PDNA TOPOISOMERS

1 - CHARACTERIZATION OF PLASMID DNA SAMPLES BY CHROMATOGRAPHIC METHODS; HERMANN SCHUCHNIGG, PATRICIA CANTARELL, CHRISTOPH POLLAK, JOCHEN URTHALER AND WOLFGANG BUCHINGER; BOEHRINGER INGELHEIM AUSTRIA GMBH, POSTER HPLC 2008, BALTIMORE, MD, USA

06
WHAT'S NEW
LENS3 MALS DETECTOR

EXPANDING THE BOUNDARIES OF LIGHT SCATTERING

LIGHT SCATTERING DETECTION HAS BECOME THE MOST WIDELY USED TECHNIQUE TO OBTAIN THE SO-CALLED "ABSOLUTE" OR TRUE MOLECULAR WEIGHT OF MACRO-MOLECULES SUCH AS SYNTHETIC POLYMERS, BIOPOLYMERS, PROTEINS, AND ANTIBODIES. WHEN COUPLED WITH SIZE EXCLUSION CHROMATOGRAPHY (SEC), MOLECULAR WEIGHT AND SIZE, AS WELL AS AGGREGATION OR BRANCHING CAN BE INVESTIGATED.

The latest light scattering technology recently introduced by Tosoh Bioscience features an entirely new detector design that addresses the limitations and shortcomings of the most popular traditional instrumentation. Historically, the first LS instruments used a low angle approach (LALS) in which the angle of measurement is close enough to 0° to assume safely that the measurement at such a low angle is the same as the theoretical 0° measurement. This is still considered the purest and most accurate molecular weight measurement, as it requires no additional assumption or extrapolation. However, at that time LALS instruments were very difficult to use and a low angle measurement alone does not provide size information since it does not measure the angular dependence of the scattered intensity.

To achieve both easier molecular weight and size measurements simultaneously, multi-angle light scattering (MALS) detectors were developed to collect light at multiple angles. These measurements are extrapolated back to 0° for molecular weight (MW) determination, while the scattering pattern is mapped to obtain size information (Rg). MALS instruments typically consist of multiple photodiodes (detectors) arranged around a circular or cylindrical flow cell. There are several, purely technical limitations to this design: The geometry offers limited space available to, physically, fit the detectors around the cell. As a result, the lowest and highest measurable angles can only be relatively far from 0° and 180°. Furthermore, due to the glass-type material of the optical flow cell, reflection of the scattered light on the cell walls creates stray light that interferes with the measurement. The resulting noise affects the lower and higher angles even more specifically, as compared to the 90° angle. Consequently, it is not uncommon that those angles become unusable and must be discarded for data processing.

To address the limitations previously listed, a completely new approach to MALS instrument design is necessary. The LenS3 MALS detector combines a novel flow channel concept (see figure below) with improved optics to provide more signal intensity while reducing the core noise at each angle of measurement. Overall, the gain in performance and sensitivity of the LenS3 MALS detectors, as compared to traditional MALS instruments, comes from the combination of the following elements:

Extreme angles of measurement

- True, usable ultra-low angle (LALS at 10°) for accurate and direct MW determination without extrapolation
- Ultra-high angle (HALS at 170°) used in conjunction with the LALS and the 90° angle (RALS) to detect the smallest difference in scattered intensity for Rg measurements of smaller molecules

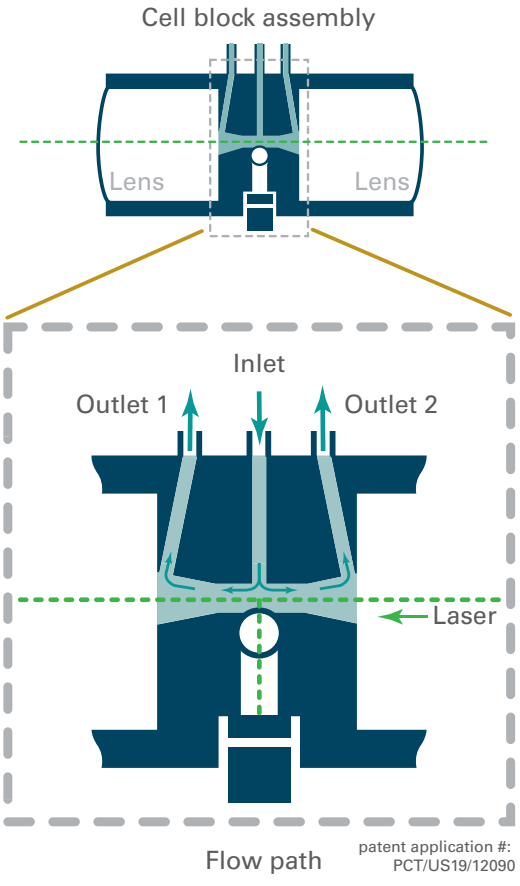
A novel flow channel

- Elongated conical shape to maximize scattering volume and thus signal intensity
- Black, inert polymeric material eliminates stray light to reduce noise

Advanced optics

- A green laser to increase the intensity of scattering by a factor of three
- Elimination of the incident beam to obtain a cleaner signal at low and high angles

This innovative approach represents the first significant advances in light scattering technologies in four decades. The instrument can detect angular dependence to an extremely low level to measure the lowest Rg ever reported by light scattering. The sum of innovations realized in LenS3 will benefit the analysis of all types of macromolecules, from biomolecules to synthetic polymers.



LENS3 FLOW PATH

07 EVENTS BIOSEPARATION FORUM

VIRTUAL BIOSEPARATION FORUM SUCESSFULLY LAUNCHED

LIKE OTHER CONFERENCES AND EVENTS, ALSO OUR RENOWNED BIOSEPARATION FORUM WAS AFFECTED BY THE PANDEMIC. THIS EVENT SERIES STARTED IN 2012 AND PROVIDED THE LATEST TECHNICAL AND SCIENTIFIC INFORMATION ABOUT SEPARATION TECHNOLOGY IN DOWNSTREAM PROCESSING AND ANALTYICS OF BIOMOLECULES. MORE THAN 400 ATTENDEES ATTENDED THESE EVENTS ALL OVER EUROPE UNTIL NOW. THIS YEAR WE DECIDED TO GO VIRTUAL BUT TRIED TO KEEP A LIVE TOUCH, BY PROVIDING LIVE MODERATION AND LIVE QUESTION & ANSWER SESSIONS.

The motto of our one day virtual BioSeparation Forum was "Gain insights into chromatography, new therapies, and antibody manufacturing & characterization". It comprised talks and scientific updates presented by specialists from Batavia, Rentschler, Roche, UGA Biopharma, Universities and Tosoh. The morning session was dedicated to manufacturing of new therapies and antibody therapeutics. The afternoon session focused on speed and accuracy in biomolecule analysis. Each section concluded with a joint Q&A session with the presenters.

The Manufacturing session started with presentations of Michael W. Wolff (University of Giessen) and Kai Touw (Batavia Biosciences) on downstream processing of viral vectors and vaccines. The antibody part of the session was opened by a key note of Alois Jungbauer (BOKU Vienna) on process intensification by parallel, flow through, and counter current chromatography. Romain Dabre (Tosoh Bioscience) and Anja Trapp (Rentschler Biopharma) presented practical examples suitable to speed up antibody manufacturing in pandemic times.

The afternoon session on analytics of antibodies focused on size exclusion chromatography (SEC) hyphenated with advanced detection such as MALS (Patrick Endres, Tosoh Bioscience) or mass spectrometry (Rob Haselberg, VU Amsterdam). Alexander Knaupp (Roche Diagnostics GmbH) presented their way to establishing a generic SEC method in a global company. Marcel Nowak 'and Stephan Schultz (UGA Biopharma) presented a new approach to quickly asses glycan profiles in process development by FcR affinity chromatography.

With over 180 registrants from all over the world this first virtual event was a big success with an overwhelming feedback. This encouraged us to continue in 2021 with more half day virtual events focused on certain target molecules such as oligonucleotides and new antibody modalities.

LIVELY DISCUSSION IN THE Q&A SESSION ON MANUFACTURING

08
WHAT'S HAPPENING
VIDEOS AND WEBINARS

#CHROMATOGRAPHY EXPERTS

DURING THE LOCK DOWN PERIOD IN SPRING WE STARTED TO RECORD THE FIRST ISSUES OF THE SERIES OF EDUCATIONAL VIDEOS AT OUR HOME OFFICES. IN SUMMER, WE COULD REALIZE THE INITIAL PLAN AND BROADCAST THE VIDEOS OUT OF OUR LARGE AND MODERN LABORATORIES AT THE EUROPEAN HEADQUARTERS IN GRIESHEIM, GERMANY.

Meanwhile three issues of our series have been completed and are available in our social media channels. The first issue explains the basics of multi angle light scattering (MALS) detection and the second issue is dealing with Fc receptor affinity chromatography that can be used to quickly assess mAb glycoforms.

The third issue was recorded this summer when the Tech Support colleagues were allowed to return to the labs. In this video Patrick Endres, Senior Laboratory Specialist, explains the ease of use of our new SkillPak 1 mL and 5 mL pre-packed columns.

Check out the video on SkillPak columns here:
bit.ly/SkillPakVideo

NEWS & EVENTS | MEET TOSOH BIOSCIENCE *IN THE VIRTUAL WORLD*

UPCOMING EVENTS

NEW WEBINAR SERIES - TOSOH TUESDAY
ENCOURAGED BY THE SUCCESSFUL FIRST EVENT OF THE VIRTUAL BIOSEPARATION FORUM (SEE PAGE 7) WE STARTED A MONTHLY WEBINAR SERIES. CHRISTIAN ROHRER, DIRECTOR SALES AND MARKETING AT TOSOH BIOSCIENCE GMBH, PRESENTED THE FIRST EDITION OF "TOSOH TUESDAY", A WEBINAR SERIES HELD BY CHROMATOGRAPHY EXPERTS ON TUESDAY, DECEMBER 1, 2020. ROMAIN DABRE, OUR SENIOR PRODUCT MANAGER, PRESENTED SOME OF TOSOH'S SOLUTIONS FOR THE EFFICIENT PURIFICATION OF BIOMOLECULES.

TOSOH TUESDAY ON JANUARY 19, 2021
THE NEXT EPISODE OF TOSOH TUESDAY WILL FEATURE CURRENT TRENDS IN THE ANALYSIS OF BIOPHARMACEUTICALS, PRESENTED BY REGINA RÖMLING.

FIND THE LATEST UPDATES ABOUT VIRTUAL AND LIVE EVENTS PLANNED FOR THE SECOND HALF OF 2021 HERE
WWW.TOSOHBIOSCIENCE.DE/NEWS-EVENTS/EVENTS