Getting a Handle on Data Handling

A panel discussion on data handling trends in the chromatography sector

Cover Story

2 Trends and Developments in Data Handling
Lewis Botcherby, Associate Editor, The Column
A snapshot of key trends and developments in data handling according to selected panellists from the chromatography sector

Features

In this instalment of “Practical GC”, air leaks in a gas chromatography (GC) system and their impact on GC stationary phases are examined.

16 Modern Size-Exclusion Chromatography Separations of Biosimilar Antibodies at Physiological pH and Ionic Strength Stephan M. Koza and Bill Warren, Waters Corporation
The state of protein-derived self-associated, aggregated, and fragmented impurities in therapeutics are critical quality attributes (CQAs) and are widely monitored using non-denaturing size-exclusion chromatography (SEC).

Regulars

8 News
The latest research news and company news

22 The LCGC Blog
Efforts to Increase Diversity and Equity in Introductory Chemistry Amber Hupp, College of the Holy Cross
The benefits of increased diversity in the workplace are clear to all, yet many chemical industries, including separation science, lack the diversity that is representative of the population.

26 Training Courses and Events

28 Staff
Trends and Developments in Data Handling

Lewis Botcherby, Associate Editor, The Column

A snapshot of key trends and developments in data handling according to selected panelists from the chromatography sector.

Q. What is currently the biggest problem in data management for chromatographers?

Christoph Nickel: Currently one of the major challenges is the increasing number of samples to run, analyses to conduct, and data to review while keeping data quality high and detecting any potential error. A major driver for this is increasingly complex separation and detection techniques that are required to analyze biotherapeutics. The result being that the chromatographer increasingly needs to use mass spectrometry (MS). Furthermore, the consolidation of all this information into an easily viewable and sharable format at a central location is a massive challenge. This is particularly important for information that is required to take an informed final review and approval.

A typical example is the weighing results for calibration standards generated from a balance that should be connected to the calibration data in the chromatography data system (CDS) for confirmation of proper calibration and eventual accurate quantitation of the unknown compounds.

Ofrit Pinco: One of the biggest challenges for chromatographers is that data from different vendors cannot be incorporated together and analyzed collectively due to a lack of a unified data format. Chromatographers can only review data from one system at a time and answer specific questions. This makes it harder to access and conduct secondary analysis across multiple data systems. To address this challenge, several pharmaceutical companies have sponsored the Allotrope Foundation (1).
whose initiative is to unify data formats. In addition, some start-ups are building tools to translate data into a common format. However, both initiatives will take some time and collaboration to overcome this challenge.

**Anne Marie Smith:** Chromatographers use a variety of different instruments from various vendors, each with their own proprietary data formats. One big problem area is bringing together and managing the data from the different electronic systems. The ability to normalize all that disparate data while retaining the ability to interrogate it, as in native data processing software, is very beneficial to chromatographers. Since chromatography data are so ubiquitous, effective management in a central, accessible place is essential.

**Björn-Thoralf Erxleben:** Handling large quantities of data requires a lot of time for data processing and interpretation. Additionally, depending on the local situation, secure data storage and archiving can be time consuming, and administration of these processes gets more and more complex.

Although there are network-based multi-instrument-capable CDSs, all vendors support and maintain their proprietary data format first—data file formats for photodiode array detectors (PDA) and for MS instruments are closed. Even when providing drivers to other CDS systems, still several requests/wishes are not satisfied. Hardware-wise, hybrid configurations may contain different operation workflows, and parameters cannot easily be transferred between vendors. Direct comparison of data between different instruments can be difficult.

**Q. What is the future of data handling solutions for chromatographers?**

**Christoph Nickel:** I see three main trends: first, radically streamlined and simplified user experience with more “fit-for-purpose” applications; second, an agglomeration of data from different sources in a single central repository in a consolidated format—often referred to as a Data Lake. This will reduce the time for data review/analysis because it eliminates any manual data transfers or manual consolidation of spreadsheets or PDF files. Third, more and more automation of routine tasks using machine learning (ML) (for routine reviews) and algorithm-assisted data mining to identify patterns, trends, outliers, or deviations.

In addition, data will continue to become available anywhere, anytime, so there will be no further need to be in the laboratory or at the instrument to analyze your data, and no need for installation and maintaining software applications on your device. Everything will be available online.

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Ofrit Pinco: The future of data handling goes well beyond acquiring and analyzing data generated by a single chromatography system. As new tools and solutions are being developed, and as researchers are being expected to extract more information from their samples, chromatographers will need to access and analyze data from multiple instruments and data systems simultaneously. Right now, chromatographers have multiple tools to help them focus on multiple areas, but future tools will allow them to review information from the whole workflow in one space. This has potential to enable researchers to answer more questions. This will also be valuable as requirements and regulations for compliance become stricter. New tools will also give research teams insight into historical instrument performance data, leading to increased operational efficiency and even predictive maintenance. Data handling will only continue to become more streamlined and more advanced through the utilization of these types of tools combined with artificial intelligence (AI) and ML. These are the next steps needed to reach the lab of the future and Industry 4.0.

Anne Marie Smith: The cloud is the future of data handling. All systems will connect to the cloud. It’s secure, simplifies the infrastructure thereby reducing costs, provides better performance, and is scalable. Depending on the system you choose, it can also be “future proof”. It is important, however, that systems architects take into account the scientists’ data access requirements. Whether the data needs to be accessed immediately upon generation, or at a later date, should inform how data management solutions are architected to ensure a seamless transition to cloud-based data access.

Björn-Thoralf Erxleben: We already see cloud-based data storage options at several CDS installations, and this trend will continue because it renders data access and sharing far easier. At the same time, this will require a new level of data security and data protection. A positive aspect is that data storage and archiving is outsourced and will not bind IT resources on-site. AI software will be implemented in “standard” software, for peak picking, processing, and identification using database packages. Self-learning algorithms will support method optimization and provide an estimation of retention time, based on structural information of the analyte.

Developing and maintaining special programs and databases for research use is a time- and resource-intensive task. If such a standard is being accepted and used in the industry, instrument vendors have to provide data compatible with these programs.
There might be also agreements about new standard data formats, which will be used or supported via conversion. Last, but not least—it would be nice to see that workflows and parameter definition is adjusted between the vendors and that data processing, at least for two-dimensional (2D) data, becomes a common piece of software accessible via the web, to be used by all chromatographers, after logging on to the dedicated cloud.

**Q. What one recent development in “Big Data” is most important for chromatographers from a practical perspective?**

**Christoph Nickel:** While it might sound boring, the biggest impact on the analytical laboratory is the ability to bring data together from all instruments and all devices working on the same function, the same research, or the same discipline. The availability of data in one location is a mandatory prerequisite for every analysis, insight, or application of algorithms. So, any effort that chromatographers can make to bring their data together brings them a major step closer to fast, efficient, and error-free analysis, moving from reactive review or error handling to proactive problem prevention. This can be realized from the availability of unlimited computing power in the cloud, which is becoming more mainstream for deployment of globally connected systems.

**Ofrit Pinco:** AI and ML have been growing rapidly in the last few years and people are realizing more of their advantages on a daily basis. Take search engines for example, Google has drastically changed the way we search for answers, plan our travels, and consume news. As AI and ML technologies mature, more scientists with this skill set will enter the chromatography field and apply these technologies to the laboratory.

In the current state, chromatographers analyze data based on specific questions, with the aim of confirming predefined hypotheses. Through AI and ML, chromatographers may be able to uncover new trends and patterns from a large set of unstructured data, giving chromatographers insights they didn’t know existed. This will greatly facilitate the progress of scientific research in the long run.

**Anne Marie Smith:** AI and ML can help find relationships and insights in an otherwise overwhelming amount of data, providing potential predicted outcomes. While AI and ML can drastically improve processes, it is only as good as the data that is input. For instance, for chromatographers where there are a multitude of possible instrument combinations, if data collection is of poor quality or incomplete, the results may be biased.

**Björn-Thoralf Erxleben:** Analytical intelligence features such as auto-recovery, start-up, self-check, and feedback. Apart from additional automation, this enables quick and easy hardware diagnostics and helps to decrease downtime of the systems.

By applying more process analytical techniques (PAT) features and more feedback from the system to the central server, chromatographers can focus on their work and need to care less for the hardware.

**Q. What obstacles do you think stand in the way of chromatographers adopting new data solutions?**

**Christoph Nickel:** One of the greatest challenges is the need to comply with good manufacturing practice (GMP) and data integrity guidelines. The validation guidelines were drafted for on-premise deployment of software, and laboratories now need to transform their validation principles into a more decentralized, globally connected world with often abstracted storage. In simple terms—the demands to prove that your data integrity is maintained now require you to include at least one additional player—the host of your data. This increases complexity of your validation tasks and requires a change in thinking and conducting validation.

Another significant obstacle is the potential delay of data access driven from the need to...
The Column

ensuring the system meets the data integrity requirements and ensuring the data are secure—and time to learn the new system. These factors often lead to reluctance to change, which can stand in the way of adoption of useful solutions.

Björn-Thoralf Erxleben: Changing an established workflow is a critical matter for analytical laboratories and operators do not always come with a strong analytical background and experience. New user interfaces, new operation workflows, and, in the worst cases, new definitions for known parameters in the software present a lot of training for users until a new solution is finally adapted. Risk of longer downtime is high. Right now, we are confronted with objection to installation of necessary service packs or patches to be compatible with modern operating systems and virus protection.

New features and functionality need to prove their advantage first before new software is rolled out and established. Another aspect is the data comparison and transfer, what happens with the “old” data? Legislations require that old data and results have to be kept and provided for inspection if needed—is maintaining a piece of the old software a good solution? Especially when it means some knowledge of how to operate it needs to be available.

Transfer the data from the laboratory to the central location/entity and access it there. While the internet and cloud performance are fast enough to provide a positive user experience, the in-house infrastructure is often the rate-limiting step. For example, a single low performance element in your local area network such as an old 10 MB switch can slow down your entire data traffic by a factor of 10. Suitability of the infrastructure is a critical prerequisite for transferring the data into the central repositories and increases dependency on your IT infrastructure.

Ofrit Pinco: A few factors contribute to this slow adoption. First is the complex laboratory ecosystem. Due to the interconnectedness of systems and solutions, any change must be evaluated for its impact on all components within the ecosystem. Also, downtime needs to be minimized, as many laboratories are in production and operate on a 24/7 schedule. After implementation, regulated labs require validation for the change. Additional training is also required for technicians to adopt new standard operating procedures (SOPs) and avoid errors. As a result, adopting new solutions is difficult and time-consuming.

Anne Marie Smith: Adopting new data solutions is a daunting task. It involves time to set up the system in a useful way, time for validation and implementation—

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Q. What was the biggest accomplishment or news in 2021/2022 for data handling?

Christoph Nickel: The adoption of the cloud with unlimited storage, computing power that enables data agglomeration, and new levels of advanced and super-fast analysis of data.

Ofrit Pinco: In the past two years, more data scientists have entered and brought changes to the analytical industry. Data scientists are skilled at analyzing and extracting insights from structured and unstructured data by using scientific methods, algorithms, and systems. They can be good complementary partners to application scientists, who have backgrounds in chemistry and understand the cases and workflows in the laboratory. Together with application scientists, data scientists can utilize models and algorithms to analyze and visualize historical data and let application scientists relate new findings to workflows and experiments.

In addition to scientific findings, data scientists may also improve laboratory operation efficiency by evaluating instrument performance and data management metrics. Data scientists may provide new perspectives on how laboratories can better store, organize, and manage data.

Anne Marie Smith: Streaming live data as it is acquired locally and storing it in a cloud instance of a CDS has improved IT systems. With the recent development in Big Data, this simplifies data movement for downstream data analytics.

Reference
1. https://www.allotrope.org

Christoph Nickel is the Director of Software Marketing at ThermoFisher Scientific.

Ofrit Pinco is Senior Product Manager at Agilent Technologies.

Anne Marie Smith is Product Manager, Mass Spectrometry & Chromatography at ACD/Labs.

Björn-Thoralf Erxleben is Senior Manager at Shimadzu Europa in charge of the pharmaceutical and biopharmaceutical market.
Thermo Fisher Scientific Expands Bioproduction Capacity in Greater Boston

Thermo Fisher Scientific Inc. (Massachusetts, USA) has opened a new biomanufacturing facility in Chelmsford, Massachusetts. The $160 million, 85,000-square-foot facility will help meet growing demand for the biologic materials needed to produce vaccines and breakthrough therapies for cancer and other diseases. This new site is part of Thermo Fisher’s $650 million multi-year investment to expand its bioprocessing production capabilities.

“This market continues to grow, and the world’s leading biopharmaceutical companies depend on our technologies, services, and expertise to help deliver life-changing therapies to patients,” said Jean Luo, VP and General Manager, Purification and Pharma Analytics, Thermo Fisher Scientific.

The technologies produced at the Chelmsford site are used in the purification process of developing biotherapeutics and vaccines. These products help save time and reduce costs so that new treatments can reach more patients faster.

“The Chelmsford site will help us provide our customers with the critical resin materials they need as they scale up production and bring new innovations to market,” continued Luo. “This ultimately helps ensure more patients get the treatments they need faster.”

When fully staffed, the Chelmsford site will employ up to 250 people and will be part of Thermo Fisher’s global bioprocessing supply network.

For more information, please visit: www.thermofisher.com

Peter Schoenmakers Wins ACS Award in Chromatography

Peter Schoenmakers of the University of Amsterdam (Netherlands) is the 2022 recipient of the American Chemical Society (ACS) Award in Chromatography. The award was presented to him at the ACS fall meeting in Chicago (USA) on 23 August.

Schoenmakers retired in June from his post as a professor of chemistry at the University of Amsterdam, where he was also the director of the van ’t Hoff Institute of Molecular Science (HIMS) and a founder and the education director of a public-private-partnership organization on analytical chemistry called “Comprehensive Analytical Science and Technology” (COAST).

Schoenmakers has made significant contributions to the field of chromatography, from his early publications on the theory of gradient elution in reversed-phase chromatography and its optimization to his work to advance the analysis of polymers, and his pioneering and ongoing work in developing two- and three-dimensional liquid chromatography (LC) methods.

Schoenmakers received his Ph.D. under Professor Leo de Galan in Delft (Netherlands) and Professor Barry Karger in Boston, Massachusetts (USA). He then he worked for Philips in Eindhoven (Netherlands) and for Shell in Amsterdam and in Houston (USA). While at Shell, he became a part-time professor in polymer analysis at the University of Amsterdam in 1998, and a full-time professor in 2002.

Schoenmakers has received multiple awards throughout his career. Recent international awards include the American Chemical Society (ACS) Award in Chromatography (2022); the Dal Nogare Award (2019); the Fritz-Pregl Medal (2018); the CASSS Award (2015); the Csaba Horváth Memorial Award (2015); the John H. Knox Medal of the RSC (2014); the Martin Medal of the Chromatographic Society (2011); and the EAS Award for Excellence in Separation Science (2010). In 2016 he received a European Research Council (ERC) advanced grant of 2.5 million Euros for the “Separation Technology for A Million Peaks” (STAMP) project.

In June, Schoenmakers was named a “Knight of the Order of the Netherlands Lion”. This royal commendation is one of the oldest and highest civil honours in Netherlands. King Willem I established the Order in 1815. Candidates who have made an exceptional contribution to society, especially in science, art, sports, and literature, are eligible for this honour.

He has also served as an editor of the Journal of Chromatography A and is a member of the editorial advisory board (EAB) of LCGC.
Researchers from “The Peppermint Initiative” have published their first results using gas chromatography–ion mobility spectrometry (GC–IMS) supporting its use in breath analysis and beginning the process of standardization of breath analysis methods (1).

The study of volatile organic compounds (VOCs) in exhaled breath has been ongoing since the 1970s, with many exhaled compounds being proposed as potential biomarkers. However, the variation in reported values is large—sometimes as high as a factor of 1000. While variability between individuals can account for some of this variation, sampling and analysis methods must also contribute heavily, adding further weight to the argument that standardization is crucial for further development and translation of breath research into clinical and deeper-research applications.

To address this issue, the International Association for Breath Research has developed the Peppermint Initiative, an international multi-centre benchmarking study seeking to provide a set of comparative data establishing a peppermint background before using a standardized dose of peppermint-oil and scheduled breath sample collections for six hours onwards. The most recent work published uses GC–IMS and the established protocols to provide benchmark values for the peppermint experiment.

The study reported five “peppermint experiments” with GC–IMS to provide benchmark peppermint washout data for this technique and support its future use in breath-testing, analysis, and research. A total of 148 samples were analyzed, with 35 ancillary tests used to evaluate the results. Twelve IMS responsive compounds were identified with eucalyptol, β-pinene, α-pinene, and limonene being the most abundant. Of those, eucalyptol proved to be the most intense exhaled peppermint-oil component, and as such, it was selected as a peppermint marker for benchmarking GC–IMS. The responses obtained by all centres provided traceable exponential washout profiles within detectable concentration levels and within a given timescale.

Interestingly, 80% of participants showed consensus in regards to the maximum exhaled concentration of eucalyptol; however, the remaining 20% showed either delayed or complex elimination profiles. This suggests some unknown factors, such as food intake and/or the effect of age, sex, race/ethnicity, or body mass, could be influencing results. The team have committed to a future study to investigate the implications of such phenotypic variability.

Overall, the benchmarking study showed how a peppermint experiment may be used with GC–IMS in different operational settings. Further studies are required to verify these preliminary benchmarks and investigate the previously mentioned phenotypic variations.

For a detailed description of the peppermint experiment, please read the introductory paper Henderson et al. (2).—L.B.

References
The Column: Hydrogen Carrier Gas for Gas Chromatography Mass Spectrometry (GC–MS)—A Practical Guide—It is a fact that most of us will be experiencing rising helium costs, and in some cases, caps on supply, or an inability to initiate new supply contracts from providers. Based on my own experiences, and those of colleagues and contacts, I’ve prepared what I hope is a succinct and focused guide on the factors one needs to evaluate to properly inform the decision to switch, and what to expect on the implementation journey. Read Here>>

Trends and Developments in Sample Preparation—A snapshot of key trends and developments in sample preparation according to selected panellists from the chromatography sector. Read Here>>

Rising Stars of Separation Science: Simona Felletti—The Column interviewed Simona Felletti, a postdoctoral researcher in analytical chemistry in the Department of Chemical, Pharmaceutical, and Agricultural Sciences at the University of Ferrara, Italy, about her work in the mass transfer phenomena and thermodynamic properties of packed columns under both LC and SFC conditions, and the hazardous potential that uncharacterized minor cannabinoids pose. Read Here>>

A Hydrophilic Strong Anion-Exchange Hybrid Monolith for Capillary Liquid Chromatography—This article presents a new hydrophilic SAX hybrid monolithic column prepared by in-capillary coating 5 μm bare silica particles with functional organic polymers. After preparation conditions were investigated and selected, the resulting column was characterized in detail. Read Here>>

Highlights from the 50th International Symposium on High Performance Liquid Phase Separations and Related Techniques—This instalment of “Column Watch” presents many of the highlighted topics and trends observed at the symposium. Read Here>>

Novel Microfluidic Chip for µSEC—Researchers have developed a novel microfluidic chip for the study of extracellular vesicles using miniaturized size-exclusion chromatography. Read Here>>

Oliver Jones Receives 2022 Barry Inglis Medal—Oliver Jones has been awarded the 2022 Barry Inglis Medal for his extended and careful development of analytical measurement techniques at the recent National Measurement Institute’s (NMI) 2022 Metrology Awards. Read Here>>

Peaks of the Month

More News

Scitara Announces Partnership With Agilent
Scitara (Massachusetts, USA) has announced a partnership with Agilent towards the integration of Scitara’s Scientific Integration Platform (SIP) with Agilent’s Software and Informatics Division portfolio of products, including chromatography software and laboratory workflow management solutions. Scitara’s SIP provides a universal connectivity solution in a cloud-native infrastructure. Data mobility plays a critical role as laboratory automation and workflow management continue to take center stage in the digital laboratory debate. The SIP integration with Agilent’s portfolio will provide fully configurable access to a wide array of laboratory instruments, applications, and resources, facilitating immediate data mobility.

“Incorporating Scitara’s SIP adds a new data integration feature for our chromatography customers, providing enhanced digital transformation through multi-directional data and workflow management. More agile data management will help our customers optimize their lab operations and increase business efficiencies,” said Thomas Schmidt, Marketing Director of Agilent’s Software and Informatics Division.

For more information, please visit www.scitara.com
Do Small Leaks in Your Gas Chromatography System Matter?

Chris English, Restek, Bellefonte, Pennsylvania, USA

Polysiloxanes (siloxanes) used for gas chromatography (GC) stationary phases are capable of withstanding repeated high-temperature cycles and injections of highly contaminated samples in a variety of solvents. Different thermal degradation pathways for siloxanes have been studied for decades, and in a dry, oxygen-free environment these materials can withstand temperatures of over 400 °C. Thermal oxidative degradation of siloxanes occurs through the bond scission of Si-O, and they subsequently form cyclic siloxanes. In addition, thermal hydrolysis also depolymerizes these stationary phases. By using a gas chromatograph mass spectrometer (GC-MS) system, the amount of water and oxygen reaching the detector relative to the tuning compound (perfluorotributyramine [PFTBA]) can be measured. In this article, the different stationary phases will be evaluated, and their resistance to oxygen and water at a variety of temperatures will be tested.

In the last “Practical GC” instalment, the origins of cyclic siloxanes in a gas chromatography (GC) system were determined by analyzing septa bleed and column bleed (1). The depolymerization of these materials results in different distributions of cyclic siloxanes. In this article, air leaks in a GC system and their impact on GC stationary phases will be examined. In a recent review article, two different types of air leaks were described: large and small (2). This is a good place to start since large leaks inhibit the ability of the instrument to operate; for example, the injection port cannot maintain pressure, the mass spectrometer (MS) will not complete a tune, or sensitivity...
will be severely compromised. These leaks can be located using an electronic leak detector or pressure decay test. In another study, helium carrier gas was purchased with 1000 µL/L oxygen added to demonstrate the effects of a leak under controlled conditions. By using a three-way valve the analyses could be performed with and without a leak. Endrin and DDT (dichlorodiphenyltrichloroethane) breakdown was measured at nearly 40% and a standard containing 1 µg/mL could not reliably be detected. Baseline instability and...
The conditions used were chosen to simulate 5% air entering the system (3). Our goal was to generate a small leak that was detectable by the GC–MS system but that would still allow the system to operate to include passing a perfluorotributyamine (PFTBA) tune.

**Experimental Design**

Two different stationary phases were chosen: a “1-type” 100% polydimethylsiloxane (PDMS) and a “1701-type” composed of 14% cyanopropyl-phenyl with the remaining 86% as PDMS (Restek). Column dimensions for both columns were 30 m × 0.25 mm, 0.25-µm film. Each column was installed into a GC–MS system (Agilent 7890 GC, 5975 MS) with a split/splitless inlet and a 100:1 split, constant flow 0.5 mL/min (1 psi @ 50 °C). The “1-type” GC program was 50 °C (hold 10 min), 10 °C per min to 200 °C (hold 10 min), 10 °C per min to 250 °C (hold 10 min), 10 °C per min to 300 °C (hold 10 min), 10 °C per min to 350 °C (hold 10 min), as shown in Figure 2. The “1701-type” GC program was 50 °C (hold 10 min), 10 °C per min to 200 °C (hold 10 min), 10 °C per min to 250 °C (hold 10 min), 10 °C per min to 280 °C (hold 10 min). Both columns were operated to their maximum programmable temperature limits with and without leaks. A 1 m × 0.25 mm internal diameter guard column was installed in the split/splitless inlet connected to the analytical column using a press-fit connector. Connections were made without a leak and with a small leak, where the small leak was determined using the mass spectrometer to scan for m/z 69 (PFTBA base peak) and compare the abundance to air (nitrogen m/z 28) and water (m/z 18). To accomplish this, a press-fit connector was used between the guard column and the analytical column. Drops of methanol were added to the connection and a leak could be observed as bubbles exiting the connector (Figure 1). Nitrogen (m/z 28) and water (m/z 18) were compared to PFTBA’s base peak of m/z 69.
to determine air and water relative to the abundance of the tuning compound and reported as percentages. To further verify that a leak was present, a leak detector and the use of methanol added dropwise to the connector was performed (Figure 1). A 1000 µg/mL pesticide standard of disulfoton, o,o,o-triethyl phosphorothioate, dimethoate, sulfotepp, methyl parathion, famphur, phorate, zinophos, and ethyl parathion dissolved in methylene chloride was used for this testing. The compounds have boiling points of between 200 °C and 400 °C. The MS scan rate was m/z 35 to 550 without a solvent delay.

Discussion
The “1-type” column used is stable to 350 °C, with variants of this phase with higher levels of cross-linking being able to withstand temperatures of 450 °C (4) using carrier gas free of oxygen and water (5). The column was installed and the system was determined to be leak-free using the mass spectrometer, a drop of methanol added to the connector (Figure 1), and a leak detector. An air and water check was performed indicating nitrogen at 1.3% and water at 0.6%. The pesticide standard was analyzed five consecutive times and the results of the runs were overlaid, indicating the column was properly conditioned—which was evident by the drop in the bleed profile and the stabilization of the baseline at the maximum programmable temperature (Figure 2). The connector was loosened and after many attempts a small leak was created, which was measured as 23% nitrogen (m/z 28) and 4% water (m/z 18) when compared to m/z 69 PFTBA GC–MS tuning compound. Notice the shift in retention time and the decrease in sensitivity.

Figure 3: 1-Type (100% Polydimethylsiloxane) stationary phase 30 m × 0.25 mm, 0.25-µm film comparing a leak-free system (black trace) to an analysis with a small leak (blue trace) measured as 23% nitrogen (m/z 28) and 4% water (m/z 18) when compared to m/z 69 PFTBA GC–MS tuning compound. Notice the shift in retention time and the decrease in sensitivity.
column to the air and water leak for 10 min at the maximum programmable temperature, the 18th analysis represented 180 min at 350 °C. In Figure 4, several representative chromatograms are overlaid, showing a steady increase in bleed, as well as an increase in the slope of the bleed, indicating damage to the column. The “1701-type” column was installed; however, a consistent leak could not be maintained and while at the maximum programmable temperature of 280 °C, the column came out of the connector and therefore an accurate bleed profile could not be obtained. Even though the column was exposed to a large leak at the maximum programmable temperature, repeated conditioning decreased the baseline bleed. Analytes eluted on average 0.15 min earlier, indicating phase loss.

Conclusions
Previous studies have evaluated larger leaks in the system and found significant loss in sensitivity, analyte breakdown, high electron multiplier voltages, and frequent filament changes, as well as source cleaning (2,3). The goal of this article was to evaluate the impact of a small leak in the system that could be measured by the mass spectrometer but would allow the system to operate to include a passing tune. Introducing a small leak measured as 23% nitrogen and 4% water caused a retention time shift—a dramatic decrease in sensitivity and measurable increase in bleed. This further strengthens the case for proper gas management and illustrates that even trace amounts of air and water will adversely impact GC analysis, especially at higher temperatures.

Acknowledgements
Special thanks to Jaap de Zeeuw and Erica Pack (Restek) for their technical advice and review.

References

Chris English has managed a team of chemists in Restek’s innovations laboratory since 2004. Before taking the reins of the laboratory, he spent seven years as an environmental chemist and was critical to the development of Restek’s current line of volatile GC columns. Prior to joining Restek, he operated a variety of gas chromatographic detectors conducting method development and sample analysis. Chris holds a B.S. in environmental science from Saint Michael’s College, USA.

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Modern Size-Exclusion Chromatography Separations of Biosimilar Antibodies at Physiological pH and Ionic Strength

Stephan M. Koza and Bill Warren, Waters Corporation, Milford, Massachusetts, USA

The state of protein-derived self-associated, aggregated and fragmented impurities in biotherapeutics are critical quality attributes (CQAs) and are widely monitored using non-denaturing size-exclusion chromatography (SEC). The mobile phases often used for accurate quantification of impurities on high sample-throughput SEC columns often deviate from the pH of the formulation buffer and can be of much higher ionic strength, which may result in the dissociation of non-covalently aggregated proteins during SEC analysis. Therefore, it may be beneficial to use phosphate buffered saline (PBS) at a physiologically relevant pH and ionic strength as an SEC eluent for the analysis of monoclonal antibody size variants (mAbs).

One of the major limitations in conducting reliably accurate size-exclusion chromatography (SEC) of various monoclonal antibody (mAb) biotherapeutic aggregates, desired monomers, and fragments is undesired secondary interactions stemming from a lack of SEC particle and/or column hardware inertness. Consequently, scientists have had to invest significant time and resources to determine the effect that SEC eluent pH and ionic strength.
strength have on these protein size variant separations. In addition, scientists have desired yet struggled to find a “generic/platform SEC eluent formulation” for SEC columns for the ultrahigh-performance liquid chromatography (UHPLC) or high performance liquid chromatography (HPLC) -based SEC analysis of various mAbs, including biosimilars and antibody–drug conjugates (ADCs).

Cross-linked dextran-agarose SEC particles (> 8.6 µm) in glass columns have been commonly used for the analysis of proteins at or near physiological conditions for over 30 years because they exhibit superbly low levels of unspecific ionic and hydrophobic interactions (1). However, the comparatively larger particle size and the compressibility of dextran-agarose SEC particles require the use of lower operating flow rates compared with the use of more rigid silica-based SEC particles. This results in longer analysis times to obtain desired mAb aggregate, monomer, and fragment resolution and quantification.

Chromatographers who perform SEC for mAb size variant analysis have frequently expressed a desire to use a single SEC eluent,
for example, a platform SEC eluent/method, to obtain accurate and reproducible data for these determinations. Ideally, the ability to use commercially available, filtered, and sterile phosphate buffered saline (PBS) would significantly reduce the time to make as well as test these different SEC eluent formulations. Furthermore, the use of PBS as an SEC mobile phase provides a pH and tonicity consistent with serum, interstitial fluid, and lymph, which are primary fluids that an intravenously or subcutaneously administered parenteral therapeutic protein is exposed to, making it a relevant SEC eluent choice. However, as shown in Figure 1, significant method development resources evaluating the independent effects of SEC eluent pH and ionic strength are frequently required to obtain the desired results of biosimilar mAbs, ADCs, and other mAb derivatives, such as bi- or tri-specific antibodies.

SEC column manufacturers have actively worked to create new SEC particle and column hardware technologies that minimize undesired secondary ionic and hydrophobic interactions between proteins and many UHPLC and HPLC SEC column offerings.
(2,3). As shown in Figure 2, the synergistic combination of BEH SEC particles bonded with hydroxy-terminated polyethylene oxide (PEO), efficiently packed in “inert” stainless steel column hardware, delivers desired mAb aggregate, monomer, and fragment component resolution throughout a wide range of SEC eluent pH and ionic strengths. The purpose of the SEC column surface hardware modification is to greatly reduce ionic interactions that occur with the formation of meta-complexes between the analyte proteins and metallic surfaces while maintaining the high efficiency and reproducible SEC particle packing capabilities obtained when using traditional stainless steel column hardware. In addition, the diol bonding of the BEH SEC particle has been supplanted by PEO to further minimize non-desired secondary hydrophobic interactions.

Method
To take advantage of the desired properties of “inert” UHPLC and HPLC column surface hardware, the separation of four commercially available biosimilars using PBS (1.15 g/L anhydrous Na₂HPO₄, 0.2 g/L KH₂PO₄, 0.8g/L NaCl, 0.2 g/L KCl, pH 7.4) SEC mobile phases at ionic strengths ranging from 150 mM (1× PBS), 120 mM (0.8× PBS), and 300 mM (2× PBS) were evaluated on 2.5 µm Waters MaxPeak Premier Protein 250 Å SEC columns. In addition, the commercially available cross-linked, dextran-agarose SEC column (Superdex 200 Increase 10/300GL, particle size >8.6 µm) (Cytiva) was also included due to the known “inert” properties of these SEC particles packed in glass columns. Examples of the mAbs that are currently available commercially in the United States as biosimilars are bevacizumab (25 mg/mL), infliximab (10 mg/mL), rituximab (10 mg/mL), and trastuzumab (21 mg/mL). All samples were analyzed neat following one or more freeze-thaw cycles that were performed to increase potential mAb aggregate formation. Note that as shown in Figure 1, a 7.8 × 300 mm, 2.5 µm mAb SEC 200 Å column (column 1) (Waters: BEH-DIOL SEC particles in stainless steel hardware) was also evaluated, and compared with separation performed on a 7.8 × 300 mm, 2.5 µm XBridge Premier Protein SEC 250 Å column (Waters: BEH-PEO SEC particles in “Inert” column hardware), to look at the influence of SEC eluent pH and ionic strength on the separation of the mAb trastuzumab.

For this work, the dextran-agarose SEC column was run at a flow rate of 0.25 mL/min to maximize resolution. Other scientists have reported that despite the low levels of protein-column interactions afforded using dextran-agarose SEC particles in glass columns, some protein size variants are not fully recovered due to unspecific ionic interactions (4).
These results indicate that the surface chemistry of the inert column hardware and BEH-PEO particles have reduced unspecific interactions with the four biosimilar mAbs in comparison with results obtained using the previous generation of BEH-diol-bonded SEC particles contained in unmodified stainless steel column hardware. More specifically, these results correlate with the literature-derived isoelectric points (pI) of these mAbs. The reported measured pI values of trastuzumab (pI = 9.1) and rituximab (pI = 9.4) are significantly more basic than bevacizumab column were approximately 63 min, 16 min, and 12.5 min, respectively.

**Results**

Consistent chromatographic profiles for the biosimilar mAbs (Figure 3) and comparable quantitative results for HMWS1 and HMWS2 (Figure 4) were obtained on the dextran-agarose SEC column, 2.5 μm BEH-PEO column and 1.7 μm BEH-PEO particles contained in “inert” stainless steel hardware, with the predominant differences in component resolutions resulting from differing SEC column efficiencies.

Therefore, these same four biosimilar mAbs were evaluated on a dextran-agarose SEC column at PBS concentrations ranging from 0.8× to 2×, with collected data demonstrating reproducible recoveries (data not shown). As a result, and in the absence of additional corroborative data such as analytical ultracentrifugation (AUC), the high-molecular-weight species (HMWS 1 and HMWS 2) content observed using the Superdex 200 Increase 10/300GL 8.6 μm SEC column was used to evaluate the effectiveness and recoveries on a 7.8 × 300 mm, 2.5 μm XBridge Premier Protein 250 Å SEC column (Waters) and a 4.6 × 300 mm, 1.7 μm Acquity Premier Protein 250 Å SEC column (Waters). The flow rate used for the 2.5 μm BEH-PEO column was 0.5 mL/min as previously described, while the flow rate for the 1.7 μm BEH-PEO column was 0.35 mL/min, which is twice the linear velocity used for the XBridge column and was possible due to the higher packed column efficiency of the 1.7 μm versus the 2.5 μm columns. As a result, the analysis times for the dextran-agarose SEC column, 2.5 μm BEH-PEO column, and 1.7 μm BEH-PEO column were approximately 63 min, 16 min, and 12.5 min, respectively.
(pI = 8.3) and infliximab (pI = 7.6), which may account for the greater degree of observed non-desired cationic interaction at pH 7.6, with low abundance negative charges such as silanol-diol or BEH-diol SEC particles in packed SEC columns (5). These results indicate that compared with many silica-diol or BEH-diol SEC particles contained in stainless steel hardware, BEH-PEO SEC particles contained in “inert” stainless steel hardware provide a broader range of SEC eluent formulations capable of delivering effective protein size variant separations (biosimilar mAbs) and, as such, should be more amenable for use in platform SEC analytical methods.

By reducing the flow rate on the dextran-agarose SEC column to 30% of the linear velocity used on the SEC column containing BEH-PEO 2.5 μm particles contained in “inert” stainless steel hardware, an effective separation of the HMWS variants was obtained, while the low-molecular-weight species (LMWS 1) fragments were partially resolved.

However, comparative run times on the dextran-agarose SEC column were increased approximately fourfold.

**Conclusion**

These results demonstrate that effective and robust high sample throughput SEC separations for four biosimilar mAbs were achieved using a 1× PBS physiological buffer (20 mM phosphate buffer with 150 mM NaCl, pH 7.4) as an SEC mobile phase on dextran-agarose 8.6 μm, BEH-PEO 2.5 μm, and BEH-PEO 1.7 μm particles contained in glass or “inert” stainless steel column hardware, respectively. While it is not expected that PBS will be an effective mobile phase for the SEC analyses of all protein size variants, the use of PBS at varying concentrations offers the potential for simpler method development, transfer, and ease of use for mAb biosimilars and other proteins where reliable and accurate size-based separations are needed.

**References**


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Increased diversity in the workplace brings broader perspectives and innovation. Yet many chemical industries, including separation science, lack the diversity that is representative of the population. To fix this problem, we must start at the beginning of the pipeline.

Understanding the Lack of Diversity in STEM

I recently read a paper in the Journal of Chemical Education that cited some statistics regarding diversity among chemists. White et al. reported that “only 6.2% of chemists and materials scientists, chemical engineers, and chemical technicians identified as Black or African American, and only 7.0% identified as Hispanic or Latinx, percentages that are far lower than in their total U.S. populations” (1). The data are not broken down by sub-discipline, but I would imagine the representation in the separations community is quite similar.

I have been reading about diversity, equity, and inclusion (DEI) in introductory science, technology, engineering, and mathematics (STEM) courses for several years now. So, I guess these numbers shouldn’t have been quite as shocking to me as they were. I know that racially minoritized students express interest in STEM at the same rate as their white peers, yet do not persist at the same rate through STEM courses and majors. In fact, national trends show that only 14.7% of...
STEM bachelor’s degrees are awarded to minoritized students (1). At many institutions, the DFW rate (the percentage of students that earn a D or F grade or withdraw from a course) in introductory STEM courses is higher for minoritized students than it is for white or Asian populations. First generation students are also more likely to feel excluded and struggle in introductory courses.

The problem has long been thought to be the students. That is, that students who have a stronger background or are highly motivated to learn can be successful, while students with weaker backgrounds may not be able to rise to the rigour of STEM courses. As Wilson-Kennedy et al. stated clearly in their 2020 article, “We need to move beyond traditional arguments of merit and preparedness to start to question how these individuals experience our institutions in the classroom, the research laboratory, and the scientific culture” (2). They argue, like many other experts in DEI practices, that we must investigate the role of bias, marginalization, systemic racism, and microaggressions in order to create an inclusive and welcoming environment in our classrooms and laboratories (2). The problem is not our students. The problem is how are students experiencing our classrooms. Equitable and inclusive foundational courses are needed for all students, regardless of preparation and background, to thrive and learn. This paper, along with David Asai’s informative manuscript titled “Race Matters”, are valuable resources for scientists wanting to learn more about structural inequities in STEM education (2,3).

Thankfully, folks in higher education are paying attention. I know from attending ACS workshops geared towards department chairs that many institutions are evaluating the inclusivity of their introductory sequences. Workshops and conferences devoted to discussion of diversity, equity, inclusion, and racism in science are now easy to find. Many institutions have DEI offices. Some institutions, like mine, have Deans of Faculty who are gently pushing faculty to evaluate their instructor mindset, their classroom ecology, and their curricular practices. Most academic chemists have little formal training in education. We learn by our own practice, by reading the literature, and through discussion of best practices with other educators. It is now time for us to take on the important work of increasing the diversity of our field by making our classrooms more inclusive and equitable.

So You’re Not an Academic…

I know many readers here are not academics. If you’ve made it this far in this blog, you might be thinking that there is no way for you to contribute to the start of the pipeline. I would argue that industrial and government chemists play very important roles in our academic world. Our students aspire to be you one day. They love to know what types of scientists exist, what kind of work you are doing, what courses they should take to get where you are. If you are reading this, especially if you belong to a group of people who are underrepresented in chemistry, I encourage you to reach out to a chemistry department at a local college or university and offer to speak with students. That old saying that representation matters is real (4). When students see someone who looks like them, with a background like them, who struggled like them, but who made it to where they want to be, that’s huge for their confidence and their drive. I would love to see more students inspired at an early age to become separation scientists. I hope you will consider my plea to engage with this young generation of budding chemists.

Examples of Inclusive Practices

So here’s the thing. Knowing the problem exists and creating a solution to it are entirely different. There is no magic wand that can be waved to completely fix the issues of systematic injustice overnight. There are, however, many reports of small- to-medium-scale changes that can begin to address equity issues in the classroom. My goal is to provide a few of the strategies that I’ve read about this year—the ones I am most excited to share and try to implement in my own courses. A quick disclaimer: I am not an expert in this area, and this list is not exhaustive by any means. For those of you in academia, perhaps some of these strategies will be new to you and you could consider their effect in your classroom. For those in industry or government sectors, I hope you glean some insight into the changing landscape of academia and how students may be trained in undergraduate courses in the years to come.

Many inclusive pedagogies rely on the idea that students must develop a positive growth mindset (the idea that intelligence is malleable rather than fixed) for themselves. Maries et al. “note that providing a growth mindset also helps with students’ self-efficacy, because it helps students recognize that anybody could be good at [science], it just takes engaging in effective practice, working with others, and getting immediate feedback” (5). Scientists and mathematicians have developed a variety of practices that could be implemented to increase equity and performance for
students regardless of their preparedness upon starting the course (6).

I have written previously about the vast body of active learning work being done by the analytical community (7). Active learning has been shown to increase exam performance while reducing persistent achievement gaps in STEM. A variety of active learning pedagogies exist and can be customized to different classroom styles and sizes. I have used active learning in instrumental chemistry courses and in general chemistry and find their use to be engaging and dynamic.

The use of low-stakes assignments has been evaluated in different ways. Eddy and Hogan assessed the value of increased course structure, comparing low and moderate course structures, using guided-reading questions, preparatory homework, and in-class activities (8). Students have more opportunities to make mistakes that are not costly to their grade, and receive formative feedback before an official assessment like an exam. Eddy and Hogan found that in the moderate course structure group, all students exhibited increased exam performance by 3.2%, yet larger gains were observed for first-generation students (an additional 3.1%) and black students (an additional 3.2%), yet larger gains were observed for first-generation students (an additional 3.1%) and black students (an additional 3.2%) and women in introductory physics (8). This is an important note.

Not only did students of colour and first generation students perform better but all students also performed better on the high-stakes assessment.

Mindset interventions regarding stereotypes have been employed in introductory physics and biology classrooms. An article by Binning et al. describes the use of an ecological-belonging intervention meant to normalize social and academic adversity (9). The intervention occurs at the beginning of the semester to establish a growth mindset in each student early on. Students learn that it is okay to struggle and that, while the course is challenging, they too can do hard work and be successful in the class, regardless of their incoming preparation. Interestingly, the intervention was most impactful on historically underperforming students in each field, that is, minorities in introductory biology, and women in introductory physics.

Mastery grading attempts to address issues related to information retention, where students can earn enough partial credit to earn a “good grade” yet not have fully mastered a single topic (10). A list of objectives are created that are evaluated over the course of the semester. Students have multiple attempts over the semester to master each objective. This type of intervention supports a growth mindset allowing students to achieve based on continued revision instead of assigning a grade based on a first assessment. The use of mastery grading has been explored more fully in the field of mathematics where some topics are not as hierarchical. Members of my own department are experimenting with the idea of using mastery grading for some topics in our first semester general chemistry course.

White et al. claim that a combination of evidence-based inclusive practices (including fostering a sense of belonging, validating students scientific identities, allowing students to make mistakes, being an intrusive instructor, cultivating relationships, and employing active learning and group work) can reduce the equity gap in introductory courses (1). Regular interventions, such as framing the challenges students may face on an exam, presenting a “Chemist of the Week” drawing from a diverse pool of chemists, and encouraging reflection of their exam performance using an “exam wrapper” exercise, were employed to increase equity in the classroom.

Muniz et al. share their efforts to transform an introductory chemistry course using a combination of cognitive and affective strategies (11). For example, students find the support of a highly structured course (cognitive), along with interleaving of course topics (cognitive), and affirmation-based mindset interventions (affective) to be transformative in their learning. The authors provide concrete examples and a detailed schedule of the cadence of both affirmative interventions and cognitive assignments.

A Final Thought

Change in academia is slow and can be quite hard at times. This work is no different. Increasing inclusivity and equity in chemistry will require a positive instructor mindset, one of growth of the discipline. It will require invention and trials. It will involve exploration. I have begun to think about this work as a summative process, that with each paper read, each intervention implemented, and each student that achieves their goals, we are inching our way to a more diverse group of chemists, analytical chemists, separation scientists. And while that work may be slow and the road long, the end goal is too important to not try.

References
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This blog is a collaboration between *LCGC* and the American Chemical Society Analytical Division Subdivision on Chromatography and Separations Chemistry (ACS AD SCSC).

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