

CATALYST Cue

VOLUME 02

ISSUE 02

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INTEGRATED APPROACHES FOR MODERN LAB WORKFLOWS

pH MADE EASY
PRACTICAL STEPS FOR ACCURACY

**THE WATER WAR NOBODY
TALKS ABOUT**

**PARTITIONING PERFECTED:
A LOOK AT CPC TECHNOLOGY**



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About Us



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Inkarp, established in 1985, honors 40 years of dedication to the scientific community in India. Our journey continues to be driven by the pursuit of excellence in **offering cutting-edge scientific research instruments**. We take pride in providing scientists across the country with tools that facilitate discoveries and innovation.



Spark Scientific, a wholly owned subsidiary of Inkarp, focuses on 4 specific market verticals - **Chromatography consumables, In-Vitro Diagnostics, Life Sciences and General Lab Instruments.**



Advion Interchim's nearly three-decade dedication to serving scientists yields customer-focused life science solutions. **Our deep scientific, engineering, and customer workflow knowledge enables an unrivaled solution portfolio.**

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Dear Readers,

Precision. It is a word we hear often in the lab. In methods. In medicine. In measurements. But here is the thing—true precision is rarely black and white. It lives in the grey. And that is exactly where this issue begins.

Our cover story, Grey Areas in the Quest for Precision, explores that tension. How do we pursue personalised medicine while maintaining uncompromised data integrity? How do we draw the line between meaningful variability and problematic noise? These are questions not just for regulators, but for every scientist designing a method, running a trial, or interpreting results.

This issue brings you two powerful features. The first shines a light on the quiet but serious battle around water quality in labs—a topic most researchers acknowledge yet rarely bring to the forefront. The other introduces WaveMode Nanomechanical Mapping (NMA), a high-speed breakthrough in AFM technology that is reshaping the way we study biological samples with unprecedented resolution and accuracy.

We also bring to you insightful conversations. First, with Dr. Zalán Kádár, RotaChrom Technologies, who shares how their scalable CPC systems are redefining downstream purification—transforming what was once seen as a niche technique into a global platform for high-purity separations. And then with Dr. Srinivas Suryapeta, co-founder of Vtides Life Sciences, who walks us through how peptide innovation is evolving in India and what it takes to build relevance in a space dominated by global giants.

Our application showcases, as always, are packed with real lab relevance—from fighting food fraud with Bruker FT-NIR, to tracking polymer behaviour using Hitachi DSC, to unravelling enzyme binding mechanisms with Reichert's Surface Plasmon Resonance. There is also a piece on improving 2D electrophoresis with high-purity water from Sartorius, and another on using ThalesNano reactors for nanoparticle catalysis in reduction and H-D exchange reactions. In our AI and Automation section, we explore the expanding universe of polymer synthesis—from emulsions to pressure polymerization—and how smart workflows are helping researchers scale output without compromising repeatability. A case study shows how Chemspeed's automated platforms are driving breakthroughs in polymer recycling—turning lab insights into scalable solutions.

The Product Highlight this time focuses on the FiveEasy™ Plus pH meter FP20-Bio-Kit, a compact, smart solution built for accuracy without the noise. It ties in neatly with our Tech Corner, which gives you practical tips to get pH measurements right—often the first step in every experiment, and sometimes the most overlooked.

And finally, we feature RotaChrom's scalable IEX-CPC method for isolating oligonucleotides, a real-time reaction monitoring story using the Advion expression® CMS, aggregate separation insights from Shodex, and small-scale peptide and impurity isolation using Waters ACQUITY UPLC and Fraction Manager.

If even one article here makes you pause, reflect, or reconsider a method you use every day, then this magazine is doing its job. As always, thank you for reading. Your time and attention mean more than we say.

Best regards,



Arun Mathrubootham
Director
Inkarp Instruments Pvt. Ltd.

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GREY AREAS IN THE QUEST FOR PRECISION

When it comes to precision, the margin of error has never been smaller.

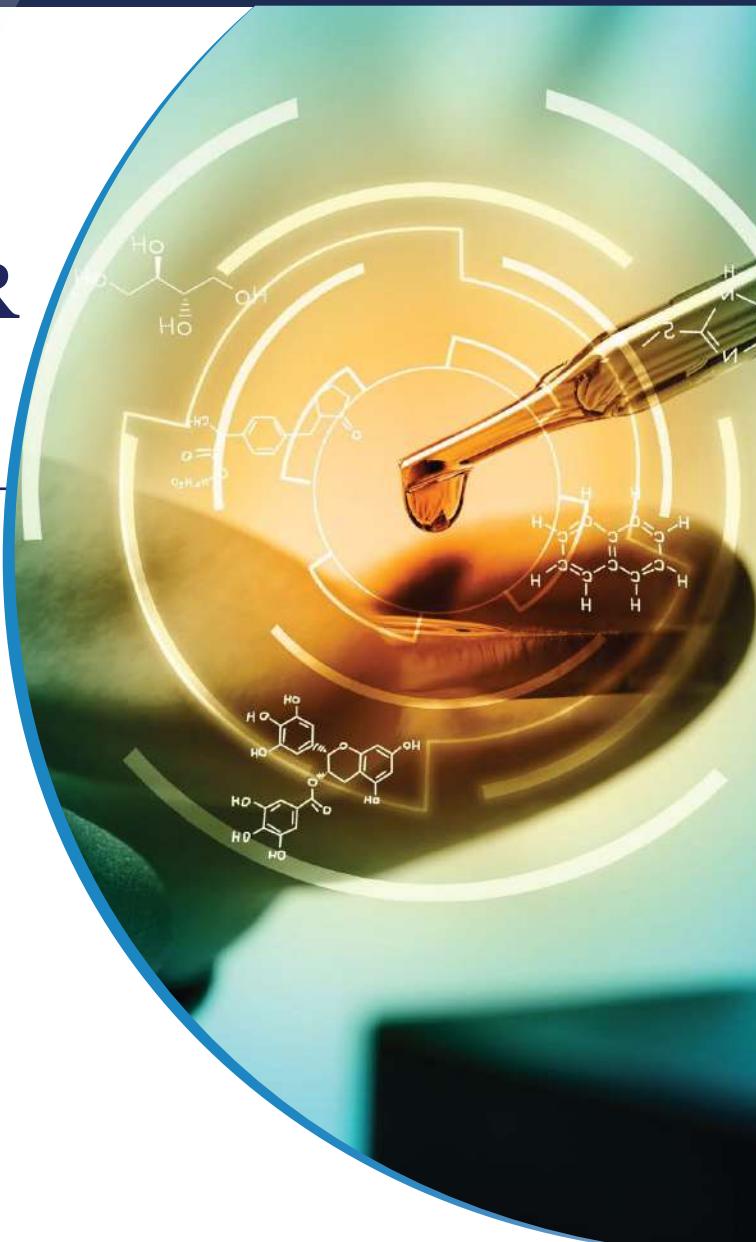
A future where clinical trials are more intelligent, results are more predictable, and treatments are customised to each patient's unique biology – is what precision medicine promises. At first look, it appears to be a scientific utopia: patients are spared from ineffective treatments, trials are optimised by algorithms, and therapies are matched to DNA. Beneath the promise, however, is a complicated reality where the boundaries of "precision" are continuously being tested by ethical conundrums, sensitive data issues, and clinical trial failures.

Drama Unfolds...

Take the example of a global cancer trial in 2023. The goal of the study was to match patients with treatments according to the mutations in their tumours. The trial gave one patient hope by offering a treatment customised to their genetic profile after traditional chemotherapy had failed.

The trial encountered an unforeseen problem halfway through. Concerns were raised by updated data protocols that the medical staff might not be the only ones to access the patient's private information. All of a sudden, concerns regarding the ownership, consent, and use of personal health information became prominent.

This situation exemplifies a fundamental paradox of precision medicine: its potential is contingent upon



detailed personal information, such as complete medical histories, biomarker profiles, and genomic sequences.

If privacy isn't protected, patients may withhold information, datasets may be incomplete, and AI-driven predictions risk bias or inaccuracy.

Precision is as much about trust and ethics as it is about science.

FAILURE: PRECISION'S UNSPOKEN REALITY

Despite its strength, precision medicine is not perfect. More than 90% of drug candidates never advance past phase III trials, even with sophisticated targeting and advanced design. At best, even trials hailed for their inventiveness, like SHIVA, which matched patients with advanced cancer to treatments based on the genomics of their tumours, demonstrated only slight progress.

What causes so many "precise" treatments to fail? Human biology holds the key: even the most well-intended treatments can be derailed by erratic tissue reactions, unanticipated toxicity, or obscure molecular interactions. Understanding the limitations of precision medicine is essential for both patients and scientists, as it is merely a tool and not a guarantee.

PROMISE AND PITFALLS OF AI

Precision medicine now relies heavily on artificial intelligence. Neural networks optimise dosing strategies, design molecules, and forecast trial results. AI can lower costs and speed up discovery, but it also brings with it new difficulties.

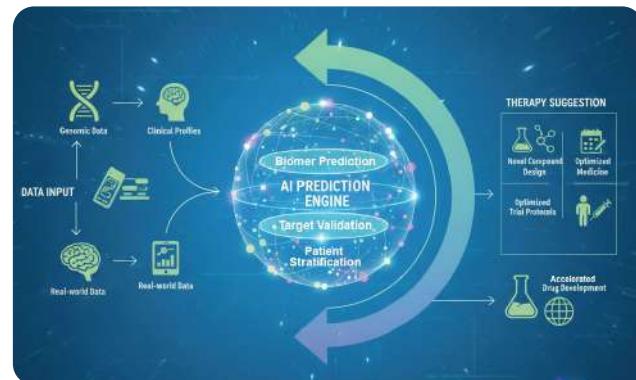
Results that are clinically or ethically flawed but scientifically believable can be produced by algorithmic bias, incomplete datasets, and a lack of transparency. Researchers' capabilities are enhanced by AI, but errors may also be amplified in the absence of strict oversight.

An industry ethicist cautions, "Innovation must be balanced with accountability." Although AI has a lot of potential, it will never be able to fully replace human morality or judgement.

THE HUMAN ANGLE

Every algorithm, trial, and genome has a human behind it. In an effort to improve care, patients give researchers access to their most private information. Their confidence is brittle, and any betrayal, whether actual or imagined, could jeopardise precision medicine's fundamental basis.

The human element is now inextricably linked to scientific innovation, as evidenced by the demands of regulators for stronger consent frameworks and patient advocacy groups for transparency. Precision medicine is not just about molecules and algorithms; it's also about relationships, ethics, and trust.



LEADERSHIP AND RESPONSIBILITY

A few businesses are leading the way. They show that precision medicine can be both responsible and successful by combining scientific innovation with robust governance. Leading trials now typically have strong data security measures, ethical consent procedures, and transparent protocols.

Trial and lab results are no longer the only indicators of success in precision medicine. How the technology is used responsibly, how patients are safeguarded, and whether trust is upheld are the true tests.

In conclusion, the pursuit of accuracy is no longer just a technical challenge—it is a human one. Scientists and physicians must grapple with responsibility, ethics, and privacy in equal measure. The future of AI, big data, and personalised therapies will not be defined by innovation alone, but by how carefully, rigorously, and transparently we wield them.

Precision medicine will ultimately be measured by the trust gained, the data respected, and the results improved, not just by the molecules created, trials finished, or therapies authorised.

DATA PRIVACY: THE HIDDEN PILLAR OF PRECISION

Although precision medicine promises personalised treatments, its accuracy is solely dependent on the completeness and quality of personal data.

Predictive models that inform treatment choices are fed by each genome, biomarker, and medical record.

Patients may withhold information, datasets may be lacking, and AI-driven insights run the risk of being skewed or deceptive if privacy isn't protected.

In other words, without trust, precision is impossible. Safeguarding confidential information guarantees both scientific precision and ethical adherence.

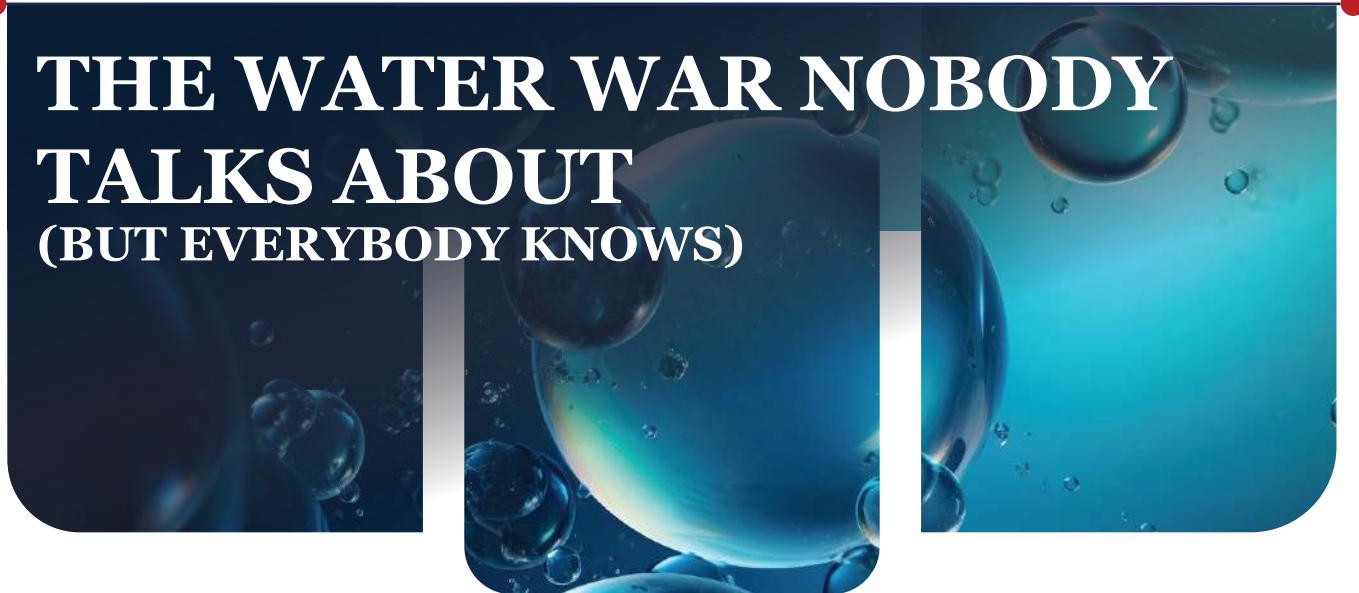
Not only does a breach or misuse jeopardise privacy, but it also obscures the resolution that enables precision medicine.



*Because your science deserves
water that works as hard as you do!*

SARTORIUS

THE WATER WAR NOBODY TALKS ABOUT (BUT EVERYBODY KNOWS)

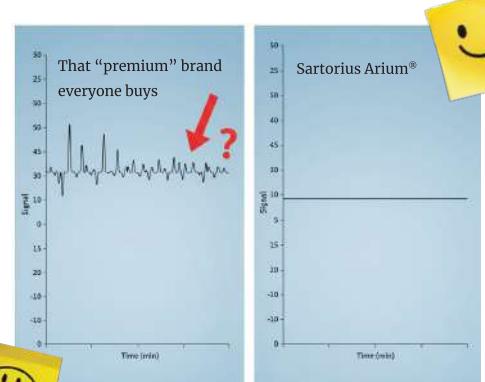


Let us be honest. In the lab, ultrapure water is that friend who never gets credit but makes every party possible. No experiment happens without it. Yet, the machines that produce it? They have become characters of their own. Some are divas (always demanding new accessories), some are the strong-silent types (reliable but boring), and then there is Sartorius WPS—cool, unfussy, and quietly outclassing everyone else.

SCENE 1: THE GREAT GHOST PEAK DISASTER

Imagine this. You run your LC-MS, waiting for that perfect clean signal. Instead, ghost peaks appear like uninvited relatives at a wedding. You blame the column, the solvent, maybe even your intern. But then you realize—it is the water system. That “premium” brand everyone buys because, well, everyone buys it. Turns out its TOC monitoring was more mood swing than measurement. Flow-rate dependent, inconsistent, and a perfect recipe for bad data.

Now switch to Sartorius Arium® Pro. TOC breakdown \leq 2 ppb. Stable. Boring in the best possible way. No drama. Just clean water and cleaner results.



SCENE 2: ERGONOMICS... OR YOGA CLASS?

Competitor systems love their fancy dispensers. Some are so bulky that using them feels like an arm workout. Others make you bend, stretch, and contort like you are secretly auditioning for yoga class.

Sartorius Smart Station? Flexible height adjustment from 8.5 to 55 cm. Left-handed, right-handed—it does not matter. Comfortable touch display. Smooth operation. Basically, a system designed by someone who actually stood at a bench for eight hours.

SCENE 3: SAFETY NETS VS. SAFETY BETS

Some systems give you a TOC “indicator.” Translation: it tells you things are fine... until they are not. Others will happily sell you an external regulator, an extra leak protector, or an upgrade pack—because apparently, safety is optional.

Sartorius flips that script. Integrated water guard? Check. Pressure regulation up to 6.9 bar at no extra cost? Check. Step-by-step maintenance guidance so you do not end up Googling “how do I not flood the lab?” at 2 AM? Also check.



DISASTER ZONE X



PROBLEM SOLVED. ✓

SCENE 4: THE CONSUMABLES CONSPIRACY

You know the story. Buy the system cheap, then spend the next five years paying through the nose for cartridges, filters, and accessories. Some cartridges even need add-on kits just to do sensitive trace analysis. Hidden costs everywhere.

With Sartorius, consumables are built to last. High-capacity ion exchange resins, activated carbon, double-layer sterile filters. Detection limits down to 0.005 ppt. That means you can trust your data and your finance department will not break into tears every quarter.

THE BIG PICTURE

When you put it all together, here is the real punchline.

- ▶ Some systems look like they belong in a museum of scientific relics (outdated menus, clumsy handling, low sensitivity).
- ▶ Others scream premium but act like subscription traps—basic safety and reliability come only if you buy “optional” extras.
- ▶ And then there is Sartorius. Simple, modern, efficient. Built for scientists, not for sales catalogs.

It is not just about ultrapure water. It is about peace of mind. No ghost peaks, no surprise bills, no ergonomic gymnastics. Just clean, consistent, dependable performance that lets you focus on your science instead of babysitting your water system.

WHY PEOPLE TALK

Here is the fun part. Scientists who switch do not just use Sartorius. They become evangelists. They joke about their old systems, share memes about clunky dispensers, and quietly admit that life at the bench just got easier. That is how Sartorius spreads—not through loud promises, but through word of mouth from people who finally stopped fighting with their water system.

FINAL THOUGHT

In the end, choosing Sartorius WPS is like upgrading from a flip phone to a smartphone. Sure, the old one technically still works. But once you taste what is possible, there is no going back.

WAVEMODE NMA: HIGH-SPEED NANOMECHANICAL MAPPING

Nanosurf developed a comprehensive suite of nanomechanical characterization methods for the DriveAFM addressing diverse research and industrial requirements. Our nanomechanics suite includes Modular Force Spectroscopy solutions for precise measurement of tip-sample interaction forces and the Viscoelastic Analysis for detailed characterization of material properties such as elasticity and viscous response across varying timescales (Figure 1).

WaveMode NMA is at the forefront of Nanosurf's nanomechanical analysis technologies. It combines photothermal off-resonance tapping with advanced nanomechanical evaluation. WaveMode NMA is the fastest commercial method for force-curve acquisition, offering unprecedented temporal resolution while maintaining exceptional force sensitivity. This technique dramatically shortens acquisition times compared to conventional force mapping approaches, allowing for collection of comprehensive nanomechanical datasets across large sample areas in previously unimaginable timeframes. The photothermal actuation at the heart of WaveMode NMA eliminates the speed limitations of traditional piezo-driven methods. By working off-resonance, WaveMode NMA delivers direct, quantitative measurements of sample properties circumventing the complexity inherent to

interpretation of non-linear dynamic analysis modalities. This overview explores the principles, implementation, and advantages of WaveMode NMA technology, demonstrating how it enables researchers to access new frontiers in materials characterization through rapid, reliable, and quantitative nanomechanical mapping.

CALIBRATION AND OPERATION IN NANOSURF STUDIO

Like every nanomechanical measurement, WaveMode NMA also requires a calibration step to obtain quantitative nanomechanical information. WaveMode NMA calibration is fully incorporated into our automatic cantilever calibration procedure, requiring only a hard reference sample like silicon, glass, or sapphire (Figure 2). This streamlines the setup process and enhances the user experience.

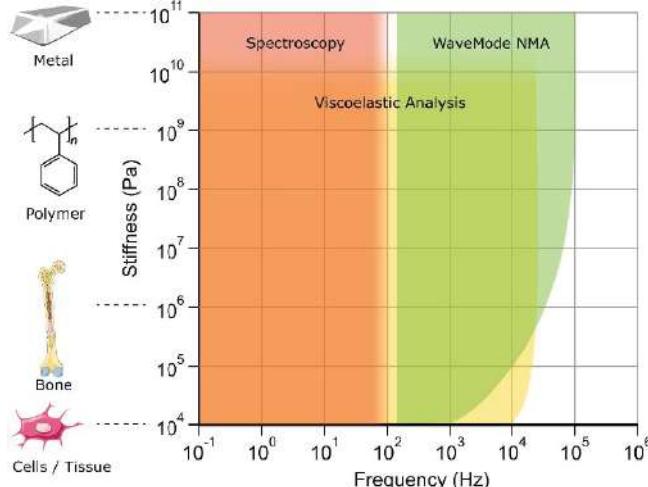


Figure 1: Nanosurf nanomechanics portfolio selection guide. The graph displays three complementary methods (classical force spectroscopy, viscoelastic analysis, and WaveMode NMA) plotted against sample stiffness and measurement frequency. Shaded regions indicate the optimal application range for each technique. To select the appropriate method, identify your sample's stiffness range and required measurement frequency, then choose the technique whose shaded area encompasses that point. WaveMode NMA offers the fastest force-curve acquisition across a wide range of sample stiffnesses.

Likewise, high-speed nanomechanical analysis is remarkably straightforward, following the same workflow as classical WaveMode imaging. This minimal learning curve allows for a quick and smooth transition towards fast high-quality advanced nanomechanical analysis in a fraction of the time required by traditional force curve based approaches.

METHOD AND EVALUATION

In WaveMode NMA, the tip position is modulated sinusoidally via photothermal actuation, ensuring a clean and reliable tip trajectory. The deflection signal precisely measures the tip position out-of-contact with the surface (the so-called free wave) as well as in contact with the surface. For accurate quantitative analysis of the tip trajectory, the actual cantilever bending shape caused by the photothermal excitation is considered. This step is crucial,

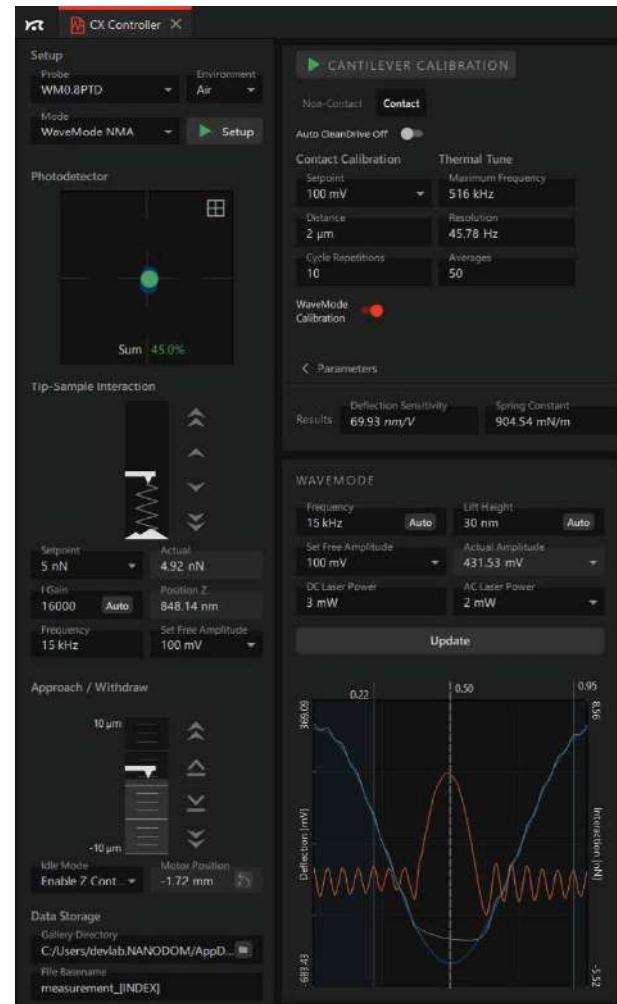


Figure 2: WaveMode NMA in Nanosurf Studio. Upper widget: The WaveMode NMA calibration process is fully incorporated in the Cantilever Calibration procedure (available from Nanosurf Studio 14 on). Lower widget: The live oscilloscope supports the user selecting appropriate WaveMode parameters.

as the cantilever trajectory is influenced by photothermal actuation. To ensure optimal WaveMode NMA performance and maximizing data acquisition speed, we carefully analyzed and optimized the usable oscillation frequency range. Consequently, the maximal operating frequency is typically capped at 10% of the cantilever's resonance frequency to prevent resonance effects. This quasi-static off-resonance operation simplifies data interpretation and analysis. The off-resonance operation is also the basis for the exceptional measurement speeds. Eventually, Wave

Mode NMA acquires force-distance curves. It thus provides rich quantitative data about sample-tip interactions. These curves can be evaluated using classical force-distance evaluation methods and various contact mechanics models to extract meaningful mechanical properties. Nanosurf Studio software offers sophisticated live analysis capabilities, allowing real-time visualization and quantification of nanomechanical properties. The software evaluates multiple nanomechanical parameters such as indentation depth, adhesion force, or Young's modulus. For the latter, multiple contact mechanics models including Hertz, Sneddon and DMT are available to accommodate different sample types and experimental conditions (Fig. 3). This versatility allows for the selection of the most appropriate model for a specific sample, whether analyzing soft biological materials, polymers, or rigid surfaces with varying adhesion properties. Immediate feedback from live analysis allows for on the-fly optimization of measurement parameters, greatly enhancing workflow efficiency while ensuring the highest quality data acquisition for advanced nanomechanical characterization.

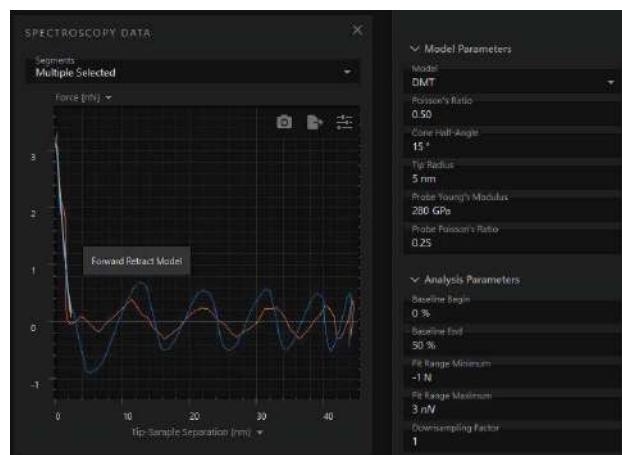


Figure 3: Analysis Tab in Studio. A WaveMode NMA interaction curve measured on an SBS sample (25 kHz WaveMode frequency) is shown together with the DMT Model fit. A selection of analysis parameters is shown on the right-hand side.

PROBE SELECTION

Nanosurf's specialized probes feature different spring constants, each corresponding to different operational

frequency ranges (Fig. 4, shaded areas) and tailored for specific fields of application and sample stiffness ranges:

WM0.1Au-SS: 5 MPa - 500 MPa

WM0.8PTD: 50 MPa - 5 GPa

WM20PTD: 500 MPa - 50 GPa

The WM0.1Au-SS probe, with its low spring constant, excels in soft matter and life-science applications where minimal sample deformation is crucial, delivering gentle yet precise measurements of delicate biological structures and soft polymers.

For more general polymer characterization and material science applications, the WM0.8PTD probe offers an ideal balance of sensitivity and robustness, operating in an intermediate frequency range suitable for a wide variety of sample types. When investigating hard samples with high elastic moduli, the WM20PTD probe delivers exceptional performance. With its higher spring constant, it provides reliable measurements on rigid materials like metals, ceramics, and crystalline structures.

This purposeful diversification of probe specifications ensures that WaveMode NMA can be optimally configured for many research applications, from soft matter characterization to advanced materials development.

CONCLUSION

WaveMode NMA represents a major advancement in nanomechanical characterization, combining photothermal off-resonance tapping with sophisticated mechanical evaluation. It is the fastest commercial method for force curve acquisition, dramatically enhancing productivity while maintaining exceptional measurement quality. The seamless integration into Nanosurf Studio software and the streamlined workflow ensure rapid adoption of this powerful technique with a minimal learning curve.

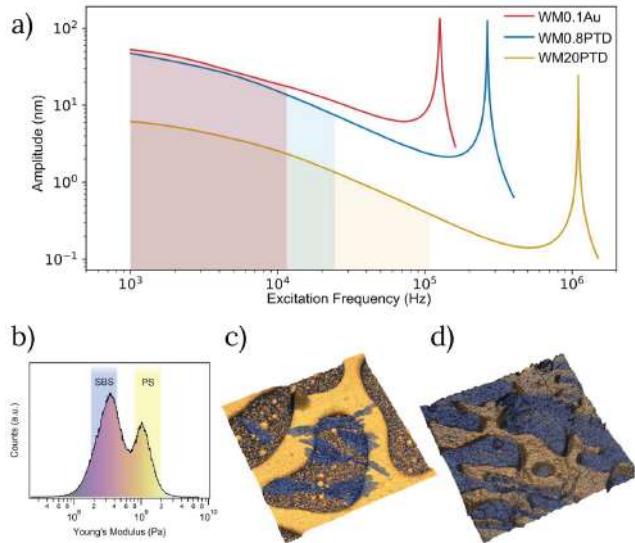


Figure 4: a) Photothermal frequency sweeps of selected WaveMode probes are shown in the upper graph. The shaded areas highlight the typical WaveMode frequency range for the respective probes. b) The histogram of the distribution of Young's Modulus values obtained from image c). c) Micro-phase separated polymer blend of polystyrene and styrene-butadiene-styrene copolymer (PS-SBS) acquired using a WM0.8PTD probe (6 μm scan size, 19 kHz WaveMode frequency, 0.1 – 3.2 GPa log color scale); d) Sn63Pb37 solder alloy acquired with a WM20PTD probe (2 μm scan size, 20 kHz WaveMode frequency, 18 – 112 GPa log color scale). WaveMode NMA maps in c) and d) are displayed as 3-dimensional topography with a Young's Modulus overlay.

WaveMode NMA leverages photothermal actuation to overcome the speed limitations of traditional piezo-driven approaches. The resulting force-distance curves can be evaluated in real-time using various contact mechanics models, providing immediate insights into sample properties. To maximize the versatility of this technology, Nanosurf has developed specialized probes with different spring constants optimized for specific applications: the WM0.1Au-SS for soft matter and life sciences, the WM0.8PTD for polymer and general material science, and the WM20PTD for hard samples requiring higher probe stiffness. In conclusion, WaveMode NMA represents a quantum leap in nanomechanical analysis, offering unprecedented speed, ease of use, and measurement accuracy across diverse research domains.

By eliminating traditional technical barriers and accelerating data acquisition, this technology enables researchers to tackle larger sample areas, conduct more comprehensive studies, and gain deeper insights into material properties at the nanoscale. Whether in academic research, industrial R&D, or quality control applications, WaveMode NMA provides a powerful tool for advancing our understanding of materials and their nanomechanical behaviour.

REFERENCE

WaveMode NMA – High-Speed Nanomechanical Mapping, (nanosurf-19956057.hs-sites.com).

 **nanosurf**



Dr. Zalán Kádár

Business Development Specialist
RotaChrom Technologies

With a PhD in organic chemistry and over 15 years of experience spanning both research and industry, **Dr. Zalán Kádár** brings a distinctive blend of scientific and strategic insight. In his current role as Business Development Specialist at RotaChrom, he focuses on expanding the adoption of CPC technology to pharmaceutical and industrial sectors.

Earlier in his career at RotaChrom as Senior Research Scientist, he dedicated his time developing preparative purification methods and representing the company's innovation at leading scientific conferences.

In the following conversation, he discusses how recent advances in CPC technology are being applied to provide scalable solutions that address purification challenges in pharma and biopharma industries.



Industrial-Scale Centrifugal Partition Chromatography

CPC had gained traction as a high-efficiency alternative to traditional chromatography. How do you see its role evolving in modern purification workflows?

I believe Centrifugal Partition Chromatography (CPC) has moved far beyond being just an alternative to traditional chromatography. It's rapidly becoming a central element of modern downstream purification strategies; particularly in pharmaceutical, biotechnological and natural extract workflows.

Since it's a liquid–liquid technique, it avoids the pitfalls of solid stationary phases, like irreversible adsorption or column degradation. More importantly, CPC scales seamlessly — from early-stage R&D right through to full industrial production — without requiring major method redesign.

As workflows advance toward continuous and high-throughput systems, RotaChrom's Continuous CPC platform, with its dual-rotor and multiple dual-mode design, fit perfectly into continuous manufacturing environments, which I think represents the future.

Could you elaborate on which types of molecules or workflows benefit most from CPC?

CPC is incredibly versatile purification solution; it can be used for a wide range of compounds. RotaChrom has already developed a huge number of applications, including, but not limited to, APIs, macromolecules and natural extracts purification. We always keep up with market trends and focus on compounds that are of great interest, such as GLP-1 receptor agonists recently.

CPC can even be applicable in truly challenging fields such as chiral separations, which are considered one of the most challenging types of stereoisomeric purification.

Our unique technology offers cost-conscious solutions for isolation/fractionation and remediation purposes, which are extremely versatile and adaptable for the realm of natural extracts.

Workflows dealing with complex natural extracts or toxic impurities removal—like pesticide or alkaloid remediation—find CPC's tailored solvent systems and robust selectivity extremely advantageous.



Moving from lab-scale to production-scale purification is quite challenging. How does CPC address scalability while maintaining efficiency and purity?

“Scalability is often one of the biggest challenges in downstream processes and this is exactly where CPC demonstrates a major advantage. **”**

At RotaChrom, we've designed platforms that make this transition as smooth and reliable as possible.

Our unique, patented cell design allows for direct and easy linear scalability across the portfolio of devices without impacting performance. The same method you develop at lab scale can be transferred all the way to industrial systems without compromising purity or yield.

The RotaChrom design allows for either a volumetric scale-up from lab-scale to industrial-scale, or even a scale-out approach, where a continuous process is employed to increase throughput. With our Continuous CPC platform, uninterrupted high-throughput purification becomes possible—delivering productivity levels that traditional chromatographic methods could never achieve.

However, scalability is not just about the technology itself, it's about the entire process. That's why we support clients with optional production-scale pilot studies to optimize operational parameters and enhance yield before committing to full-scale manufacturing.

We also provide factory design consultancy, working together with clients to design the most effective and viable plant layouts, always tailored to their processes, safety requirements and regulatory standards.

On the engineering side, we incorporate high-end CFD-assisted product development, FEED methodology to maximize output, and process simulation tools to identify the best solutions and layouts early on.

Each system also comes with an individually designed control platform, ensuring precision, reliability and ease of operation at scale.

Peptides and complex biologics are becoming increasingly important. What advantages does CPC offer in this space?

Peptide purification is one of the most challenging areas in separation science. With HPLC, you often face issues like poor solubility, co-elution of impurities, or even peptide degradation during long runs. Sticky or hydrophobic peptides, in particular, tend to adsorb onto solid supports, which leads to yield losses and inconsistent recovery.

This is where CPC really makes a difference. By using two immiscible liquid phases instead of a solid column, peptides don't get irreversibly adsorbed. Furthermore, the biphasic solvent systems are easy to tailor to match the polarity of different peptides, so you can fine-tune selectivity without being limited by column chemistry. Most importantly, CPC preserves the structural integrity and bioactivity of sensitive peptides and biologics under mild operating conditions. In many cases, CPC can serve as an efficient pre-purification step before HPLC, or even replace it, delivering fractions that are already close to final purity. This reduces the number of downstream polishing steps and accelerates the overall process.

“

All in all, for peptides and other complex biologics, CPC offers high recovery, lower costs, easier scalability and a gentler workflow.

”

CPC reduces solvent use and waste compared to traditional chromatography. How do these advantages impact both operational costs and environmental sustainability?

CPC is a “green chemistry ready” solution, which is perhaps the area we are most proud of. CPC's liquid-liquid separations eliminate the need for costly and polluting silica resins, significantly reducing consumable costs. Moreover, vast majority of the solvents can be recycled and recirculated back into the system. This not only lowers operational costs but also reduces the environmental impact, aligning with sustainability goals.

RotaChrom Continuous CPC platform takes this even further. By enabling continuous processing, we significantly increase productivity while reducing relative solvent consumption, raw material requirements and downtime. In practice, that means a much larger amount of material can be processed in the same timeframe compared to batch operations. Because of this higher throughput, it's often possible to work with more dilute solutions, which might avoid the need for in-line solvent removal or evaporation steps. Continuous chromatography enables higher throughput, improved solvent utilization, and reduced waste generation, making it a more sustainable and cost-effective solution.

I believe CPC's eco-friendly profile will be one of its most important long-term advantages as downstream processing continues to evolve toward greener, more sustainable practices.

Could you explain to our readers how labs can integrate CPC seamlessly into existing workflows?

I would say integration is actually quite straightforward. We typically recommend starting with our CPC Modeler, which allows labs to optimize their solvent systems and methods quickly and cost-effectively at bench scale.

Once the method is validated, it can be scaled up with our rCPC for pilot-scale work and then transferred directly to our iCPC or Continuous CPC systems for industrial production.

This enables straightforward technology transfer from R&D to manufacturing, which minimizes the need for method re-design and reduces overall process complexity.

“ The beauty of CPC is that you don't have to reinvent your workflow at every stage—the method remains consistent, so the transition is smooth, efficient, and compliant with regulatory standards. **”**

What differentiates RotaChrom from other CPC platforms in terms of performance, flexibility, and user-friendliness?

RotaChrom differentiates itself through a combination of advanced technology, scalability, and a real focus on compliance and automation. I usually highlight three main points. First is **scalability**. We are the only company offering a complete platform that takes you from benchtop development all the way to iCPC, the world's largest industrial CPC system.

Second is **innovation**. Beyond our CPC Simulator—which leverages a digital library of over 16,000 data entries to accelerate method development and predict outcomes—we are constantly improving the core components of the technology itself. Innovations in areas like rotary unions and cell design allow us to deliver even greater reliability, efficiency and precision with every generation of our systems.

Third is our **Continuous CPC platform**, which enables continuous injection and uninterrupted processing. This takes productivity to an entirely new level by combining

throughput, solvent efficiency and consistency in a way that batch systems simply can't match.

On top of that, we focus heavily on user experience. Our platforms are GMP-compliant, safety-certified and built to be both robust and easy to operate. Our team advises on how CPC fits into the broader purification chain, from upstream considerations to downstream integration, always with the goal of making workflows more streamlined, compliant and cost-effective. These elements make RotaChrom not just a technology provider but a long-term partner in our clients' success.

Lastly, for labs exploring CPC for the first time, what guidance would you give to maximise efficiency, and ROI?

My advice is to take a stepwise approach. I mean, start with a feasibility study to determine if CPC is the right solution for a particular application.

So, first leverage the CPC Modeler for method discovery and ideal solvent system selection—quickly and without high costs. From there, move up to rCPC for pilot work, which gives you confidence before scaling to industrial production.

Always monitor your green metrics: how much solvent you're saving, how much waste you're avoiding and what that means for your bottom line. Moreover, don't hesitate to rely on our team — we have deep expertise in both science and engineering, and we're here to support our customers through every stage of the journey. When they follow this path, they will not only maximize efficiency but also see a clear and fast return on investment.





Dr. Srinivas Suryapeta

Head - R&D, VTIDES Life Sciences,
Hyderabad

Dr. Srinivas Suryapeta, Ph.D., is an accomplished researcher and industry leader with more than 16 years of experience in the development of complex molecules, active pharmaceutical ingredients (APIs), and advanced peptide therapeutics. He is currently the Head of Department at VTIDES Life Sciences, Hyderabad, where he leads innovation in peptide synthesis, oncology product development, and technology transfer.

Throughout his career, Dr. Srinivas has held senior positions at MSN Laboratories, Alembic Pharmaceuticals, Biocon, and Troikaa Pharmaceuticals, contributing to multiple successful projects in solid- and liquid-phase peptide synthesis, process development, and regulatory submissions. His doctoral research at JNTU Hyderabad focused on the synthesis and biological evaluation of indolopeptide-based triazoles, work that has yielded high-impact publications and recognition in medicinal chemistry. He is also the inventor on a U.S. patent related to Glatiramer Acetate characterization. Renowned for his technical expertise, leadership, and commitment to advancing drug discovery, Dr. Srinivas continues to play a pivotal role in shaping the future of peptide-based therapeutics.

Peptides at the Edge of Innovation: VTIDES' Story



Q: VTIDES has positioned itself as a specialist in complex peptide synthesis. What inspired the founding vision?

VTIDES was established with the strategic vision of pushing the boundaries of peptide-based therapeutics through specialized expertise in the synthesis of structurally complex peptides. Recognizing the inherent limitations of conventional solid-phase peptide synthesis (SPPS) in producing long-chain, highly hydrophobic, or conformationally constrained sequences, the company focuses on delivering high-purity, custom-designed peptides tailored for applications in drug discovery, preclinical development, and translational biomedical research. By integrating advanced synthetic methodologies with rigorous analytical characterization, VTIDES seeks to enable the development of next-generation peptide therapeutics and research tools.

Q: Can you elaborate on your core therapeutic focus?

Our core therapeutic focus lies in the development and synthesis of complex peptides for applications in oncology, metabolic disorders, and infectious diseases. We specialize in producing bioactive peptides, stapled peptides, and peptide-drug conjugates that target protein–protein interactions and other challenging biological pathways, supporting both early-stage research and clinical development.

Q: What unique challenges come with synthesizing long, labelled, or post-translationally modified peptides and how does VTIDES tackle them?

VTIDES specializes in synthesizing complex peptides, including long sequences, labelled peptides, and those with post-translational modifications (PTMs). These peptides often present challenges such as poor solubility, aggregation, and incomplete reactions during synthesis. To address these issues, VTIDES employs advanced solid-phase peptide synthesis (SPPS) techniques, optimizing coupling protocols and utilizing orthogonal protecting group strategies. Additionally, the company integrates real-time monitoring and rigorous purification methods, including reverse-phase high-performance liquid chromatography (RP-HPLC) and mass spectrometry, to ensure high yield, structural integrity, and analytical purity of the synthesized peptides.

Q: Your facility is equipped with high-end tools like the CEM Liberty Blue, IMS-QTOF, and 21 CFR compliant systems. How do these technologies shape your innovation pipeline?

Our high-end infrastructure, including the CEM Liberty Blue, IMS-QTOF, and 21 CFR compliant systems, empowers VTIDES to deliver complex peptide products with speed, precision, and regulatory confidence. The Liberty Blue accelerates synthesis cycles while maintaining high coupling efficiency, even for long or modified peptides. IMS-QTOF offers advanced analytical capabilities for detailed mass and structural validation. Integrated with 21 CFR Part 11 compliant systems, our workflow ensures full traceability, secure data management, and readiness for clinical and commercial-scale projects—driving innovation from lab bench to regulated environments.



Q: What role does Quality by Design (QbD) play in your process development for peptides?

Quality by Design (QbD) is a foundational approach in VTIDES' peptide process development, ensuring the systematic integration of quality throughout the entire lifecycle of peptide synthesis. By identifying and controlling critical quality attributes (CQAs) and critical process parameters (CPPs), QbD enables us to optimize and standardize production processes for consistency, reliability, and scalability. This methodology incorporates risk management, design of experiments (DoE), and real-time process monitoring to proactively address potential variabilities, ensuring high-quality, reproducible peptides. As a result, QbD not only enhances product integrity but also supports regulatory compliance and facilitates efficient scale-up.

Q: Peptides are gaining momentum in cancer and antimicrobial therapies. How is VTIDES contributing to these rapidly evolving domains?

VTIDES is significantly advancing peptide-based therapeutics in oncology and antimicrobial resistance. In oncology, we specialize in the design and synthesis of peptides that selectively target tumor-specific antigens and modulate key signalling pathways, including protein-protein interactions and immune response mechanisms, thereby offering highly targeted cancer therapies. In the field of antimicrobial resistance, we focus on developing peptides with distinct mechanisms of action, such as disrupting microbial membranes or inhibiting critical microbial functions, to combat resistant pathogens. Through our expertise in complex peptide synthesis and the strategic incorporation of post-translational modifications, VTIDES is contributing to the development of innovative, targeted therapies in these rapidly evolving domains.

Q: What therapeutic areas or disease indications are seeing the most traction in peptide-based interventions?

Peptide-based interventions are gaining substantial momentum across several therapeutic areas, with notable advancements in oncology, metabolic disorders, autoimmune diseases, and infectious diseases. In oncology, peptides are being developed to target specific tumor

antigens and modulate immune checkpoints, providing potential for more precise cancer treatments. In metabolic disorders, peptides are utilized to regulate key physiological processes such as insulin secretion, glucagon regulation, and appetite control. For autoimmune diseases, peptides are being engineered to modulate immune responses and reduce inflammation. Additionally, in the field of infectious diseases, peptides are emerging as powerful agents against antibiotic-resistant pathogens, with applications ranging from antimicrobial peptides to antiviral therapies. These therapeutic areas highlight the growing role of peptides in addressing complex, unmet medical needs.



Q: Are you exploring novel delivery systems or peptide conjugates for better bioavailability?

Yes, VTIDES is exploring cutting-edge delivery systems and peptide conjugates to enhance peptide bioavailability and optimize therapeutic outcomes. We are focusing on the development of peptide-drug conjugates (PDCs), which integrate peptides with potent therapeutic agents to enhance specificity and reduce systemic toxicity. Additionally, we are investigating advanced delivery platforms, including nanocarriers, lipid-based formulations, and bio responsive systems, to improve the stability, absorption, and tissue-targeting capabilities of peptides. These novel approaches aim to address challenges such as poor solubility, rapid degradation, and limited bioavailability, thereby advancing the clinical potential of peptide-based therapies.

Q: With a strong QMS and ISO 7 & 8 GMP areas, how do you ensure regulatory readiness for global markets?

VTIDES ensures regulatory readiness for global markets through the implementation of a robust Quality Management System (QMS) that adheres to industry standards and regulatory requirements. Our ISO 7 and ISO 8 GMP-compliant facilities are designed to maintain stringent control over environmental conditions, ensuring the highest standards of product quality and consistency. We employ comprehensive risk management strategies, thorough documentation practices, and continuous process validation to guarantee compliance with global regulatory frameworks, including FDA, EMA, and other regional authorities. Additionally, our QMS facilitates traceability, audit readiness, and data integrity, enabling seamless regulatory submissions and accelerating the transition of peptide-based therapeutics from development to commercial scale.

Q: How do you align your quality policy with changing customer expectations and international regulatory standards?

At VTIDES, we ensure our quality policy remains aligned with evolving customer expectations and international regulatory standards by adopting a flexible and proactive approach. Our Quality Management System (QMS) is continuously updated to comply with the latest global regulations, including ICH, FDA, and EMA guidelines. We prioritize customer-centric

quality by integrating client feedback into our processes and maintaining open communication channels. Through ongoing training, process optimization, and a focus on continuous improvement, we guarantee that our peptide therapeutics consistently meet and exceed customer requirements while adhering to the highest standards of safety and regulatory compliance.

Q: What role do you envision peptides playing in the next wave of precision medicine or targeted therapies?

Peptides are expected to play a pivotal role in the next generation of precision medicine and targeted therapies, owing to their high specificity and ability to modulate molecular targets with remarkable precision. By targeting specific receptors, enzymes, and intracellular signalling pathways, peptides can be engineered to interact with disease-associated biomolecules, offering highly selective therapeutic interventions with minimal off-target effects.

Q: How do you see the Indian peptide CDMO space evolving, and where does VTIDES fit in this landscape?

VTIDES is strategically positioned within this evolving landscape, leveraging its advanced capabilities in complex peptide synthesis, process optimization, and regulatory compliance. With its ISO 7 and ISO 8 GMP-certified facilities and a strong Quality Management System (QMS), VTIDES is well-equipped to meet the growing demand for high-quality, tailored peptide solutions. By focusing on cutting-edge technologies, VTIDES is poised to contribute significantly to the global peptide CDMO market while supporting the development of innovative therapeutics for global markets.





FIGHTING FOOD FRAUD

Bruker FT-NIR for Targeted/Non-Targeted Adulterant Screening

This application note shows how hierarchical spectral analysis using Bruker FT-NIR spectroscopy and OPUS MultiEvaluation software simplifies food quality control. The technique allows for accurate assessment of both raw and final products as well as quick detection of adulteration by combining identification, conformance testing, and quantification into a single automated workflow. Its preparation-free, non-destructive method offers strong protection against food fraud in addition to operational efficiency.

Keywords or phrases:

Food fraud detection, FT-NIR spectroscopy, adulterant screening, quality control, non-targeted analysis, Bruker OPUS MultiEvaluation

INTRODUCTION

Maintaining brand reputation and product quality has led to increased focus on qualifying raw materials and ingredients used in food production. In addition to traditional quality parameters like moisture, fat, and protein, detection of adulterants plays a critical role in ensuring food safety and quality. The USP (US Pharmacopeial Convention) Food Fraud Database currently lists hundreds of incidents of economically motivated adulteration (EMA), substitution, counterfeiting or mislabelling of food products, such as olive oil and milk powder and some prominent adulterants e.g. melamine.

FT-NIR offers a valuable tool for screening almost any raw material with an excellent cost-benefit ratio and unrivalled ease-of-use. The high information content of NIR spectra provides a fingerprint of the complete sample.

Comparing the spectra of the incoming raw materials with

those measured using samples of known quality permits a non-targeted screening of adulterants or contaminants within the detection limits. If a material is tested positive on adulteration by FT-NIR, further investigations with complementary analytical methods can be carried out to determine the exact identity of the adulterant.

Method

Bruker FT-NIR spectrometers offer a fast and effective tool for quality control of raw materials, intermediate products and final products. As a non-destructive method without any sample preparation, it is already extensively used in the food industry for analysing main constituents such as protein, moisture, fat, lactose, ash, and fiber.

With the measurement of a single spectrum the sample can be evaluated in a three-step process. This enables verification of the sample identity and targeted screening for known contaminants as well as non-targeted screening for adulteration with unknown materials, followed by traditional quantification of the main constituents:

Identification

Identification of a sample is carried out to determine if the spectrum of an incoming raw material fits within the statistical population of authentic and previously accepted batches. With this first step it can be checked if the correct raw material was delivered and properly labelled to avoid usage of the wrong ingredient in production.

2. Conformity

In the next step the sample is further qualified using conformance test, which is a more specific evaluation of the spectrum. Each data point of the NIR spectrum is subject to a dedicated test with an individual threshold.

3. Quantification

During the quantification of the different constituents an outlier test based on the Mahalanobis Distance is performed. Again, the analysis spectrum is compared to the sample population in the individual quantification models.

CONCLUSION

Bruker Optics comprehensive spectroscopy software OPUS provides powerful tools for identification, qualification by conformity test and quantification of raw materials and finished products. The powerful MultiEvaluation package combines these individual evaluation tools to a single routine.

This allows the hierarchical evaluation of spectra using different methods, like identification of a product in a list of hundreds of products, followed by a dedicated conformity test and/or quantification of the identified product.

The software user interface links measurement and evaluation methods to a single raw material or product.

The operator only needs to select the product to be analyzed and the correct measurement and evaluation methods are performed automatically. Green checkmarks are used for passing evaluations, whereas a red cross indicates the sample has failed the test.

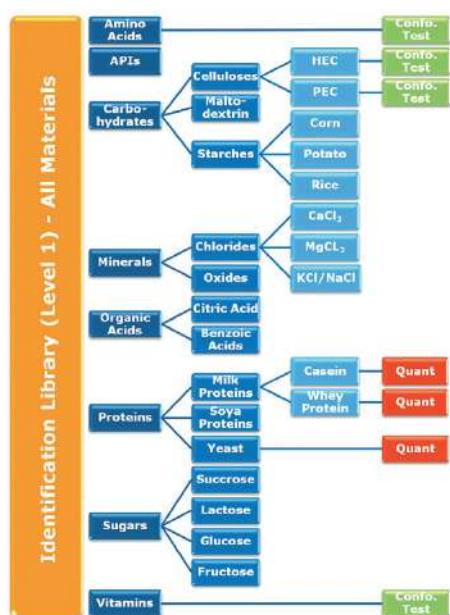


Figure 1. Example scheme of a MultiEvaluation method with hierarchical identification followed by conformity testing or quantitative evaluations.

Figure 2. 3D scores plots showing different material groups in an identification library.

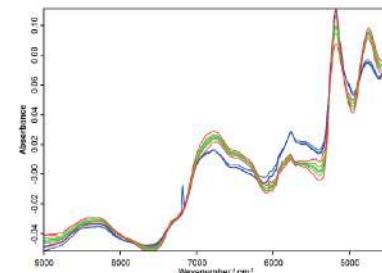
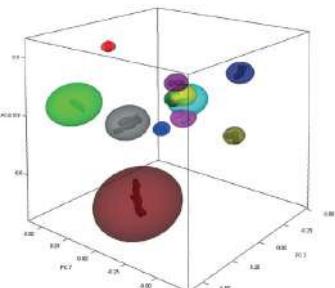


Figure 3. Display of reference spectra in a conformity test method (green) with spectra detected as outliers (blue).

Figure 4. Result screen for a quantification showing a Mahalanobis outlier for the fat evaluation.



TANGO FT-NIR Spectrometer

Touch-screen operated FT-NIR analyser for routine use in the lab and at-line.



NanoPhotometer® NP80

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Features

- Broad Detection Range*
- dsDNA: 1 – 16,500 ng/µl | BSA: 0.03 – 478 mg/ml
- Bandwidth better than 1.5 nm and
- Robust instrument design without the need for routine calibration checks
- Standalone or Remote Operated Windows, Mac, Android, and iOS
- Portable with Battery Option Only 20 x 20 x 12 cm footprint
- 5-Star Customer Support Live, application support 24/5
- GMP, GLP, GxP Ready 21 CFR Part 11 and IQOQ option*

*Cuvette detection range dsDNA: 0.1 – 130 ng/µl, BSA: 0.003 – 3.7 mg/ml

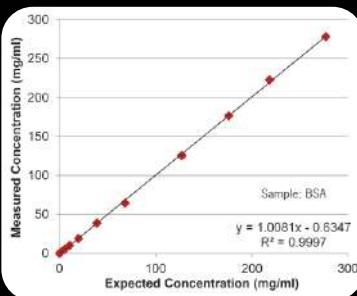
Benefits

- Broad Detection Range*
- dsDNA: 1 – 16,500 ng/µl | BSA: 0.03 – 478 mg/ml
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*Cuvette detection range dsDNA: 0.1 – 130 ng/µl,
BSA: 0.003 – 3.7 mg/ml



Detection Range	Min	Max
dsDNA	1 ng/µl	16,500 ng/µl
BSA	0.03 mg/ml	478 mg/ml



DSC Measurements of Polystyrene

Studying effects of molecular weight on glass transition using Hitachi NEXTA® DSC

This application note demonstrates the use of high-sensitivity differential scanning calorimetry (DSC) to investigate the glass transition behavior of monodisperse polystyrene (PS) samples across a broad range of molecular weights. By systematically analyzing eight PS standards, clear trends were observed in the dependence of glass transition temperature (T_g) on molecular weight, with diminishing variation at higher chain lengths.

Keywords or phrases:

Polystyrene (PS), molecular weight, Glass Transition Temperature (T_g), Differential Scanning Calorimetry (DSC), polymer characterization, thermal analysis

INTRODUCTION

Polystyrene (PS, Figure 1) is an all-purpose resin used in a variety of fields.

One structure factor that greatly influences the physical properties of PS is molecular weight. Increasing molecular weight produces rigid and strong material but it lowers workability so the appropriate molecular weight depends on how the material will be used. The weight-average molecular weight (M_w) of the commercially available PS that is widely used as industrial material is generally 150,000 to 400,000.

On the other hand, the glass transition of polymers corresponds with the start of the translational motion of chain segments, and the glass transition temperature (T_g) is essentially independent of molecular weight. However, it is known that in the molecular weight range where the degree of polymerization is low, the effect of chain ends lower T_g (1).

In this brief, DSC is used to measure T_g of monodisperse PS with different molecular weights. The 8 monodisperse PS

samples measured had comparatively low molecular weights, with weight-average molecular weights ranging from 1940 to 95,000.

MEASUREMENTS

The measurement samples were standard monodisperse PS of 8 different molecular weights manufactured by American Polymer Standards. Table 1 shows the weight-average molecular weight (M_w), number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n).

For the measurements, a NEXTA DSC200 High-sensitivity Differential Scanning Calorimeter was employed.

The sample weight was 10mg. The temperature was raised from room temperature to 160°C at 10°C/min during measurements.

Sample	M_w	M_n	M_w/M_n
PS 1	1940	1690	1.15
PS 2	4380	3570	1.23
PS 3	5480	5200	1.04
PS 4	12600	12000	1.05
PS 5	35100	30500	1.15
PS 6	65000	63700	1.02
PS 7	275000	258000	1.07
PS 8	950000	925000	1.03

Table 1. The Molecular Weights and Molecular Weight Distributions of the PS Samples

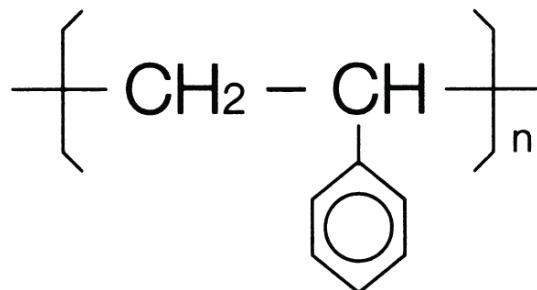


Figure 1. Chemical structure of PS

RESULTS

Figure 2 and Table 2 show the DSC measurement results for the 8 PS samples. The results show that as the molecular weight of PS rose higher, the Tg shifted higher, and the width of the shift became smaller.

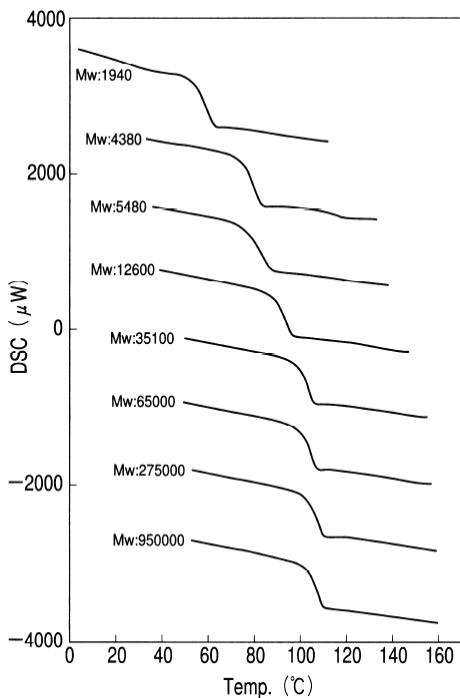


Figure 2. DSC Measurement Results for the PS Samples

Sample	Mw	Tg (°C)		
		Tig ¹	Tmg ²	Teg ³
PS 1	1940	56.8	60.4	64.2
PS 2	4380	76.2	79.9	83.7
PS 3	5480	77	82.4	87.8
PS 4	12600	89.6	93.4	96.7
PS 5	35100	100.2	103.1	105.9
PS 6	65000	100.6	104	107.3
PS 7	275000	103.5	106.4	109.4
PS 8	950000	103.9	106.7	109.7

Table 2. The Glass Transition Temperature (Tg) Measurement

Results for the PS Samples

1 Onset temperature of Tg

2 Mid-point temperature of Tg

3 End temperature of Tg

The research regarding the relationship between PS molecular weight and glass transition²⁻⁷ has been widely reported in the past. P.Claudy, J.M.Letoffe, Y.Camberlain and J.P.Pascault⁷ used DSC to investigate the relationship of the degree of polymerization of PS and glass transition.

T.G.Fox and P.J.Flory^{2,4}

$$\begin{aligned} Tg &= Tg^\infty - \frac{m}{n} (Tg^\infty - Tg^m) \\ &= Tg^\infty - \frac{A}{M_n} \end{aligned} \quad (1)$$

K.Ueberreiter and G.Kanig³

$$\frac{1}{Tg} = \frac{1}{Tg^\infty} + \frac{m}{n} \left(\frac{1}{Tg^m} - \frac{1}{Tg^\infty} \right) \quad (2)$$

P.R.Couchman⁶

$$\ln Tg = \frac{n \Delta C_p^\infty \ln Tg^\infty + m (\Delta C_p^m \ln Tg^m - \Delta C_p^\infty \ln Tg^\infty)}{n \Delta C_p^\infty + m (\Delta C_p^m - \Delta C_p^\infty)} \quad (3)$$

Figure 3 plots the relation of glass transition temperature (Tg) and the weight-average molecular weight (Mw) using the following results: the measurement results from this experiment (see Figure 2 and Table 2, the DSC measurement results of P. Claudio et al.⁷, and, as an example, results calculated using theoretical formula of T.G. Fox et al.^{2, 4}. The results presented in this brief correlate well with the DSC results of P. Claudio et al.⁷) Furthermore, P. Claudio et al.⁷ reported that, at degrees of polymerization of 450 and higher (Mw: 46800), Tg is roughly constant and the same result was found in the experiment presented here.

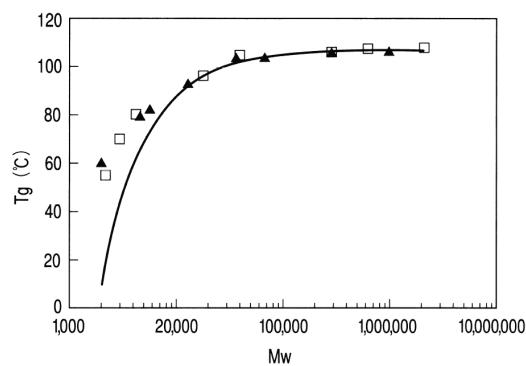


Figure 3. Dependence of Glass Transition Temperature on Molecular Weight

▲ Measurement results from this experiment (Figure 2 and Table 2)

□ Measurement results of P.Claudy et al.

— Results calculated from the theoretical formula of T.G.Fox et al.

CONCLUSION

In this application, DSC was used to measure the glass transition temperature of polystyrene with different molecular weights.

The investigation of 8 monodisperse PS samples with weight-average molecular weight (Mw) ranging from 1940 to 95,000 showed that the higher the molecular weight, the higher the glass

transition temperature. Furthermore, the measurement results presented here correlate well with the results of P. Claudy et al.⁷.

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Differential Scanning Calorimeter (DSC)

NEXTA® DSC200



Changes in the sample status can be displayed in “real time.”

Real View® Sample Observation Unit

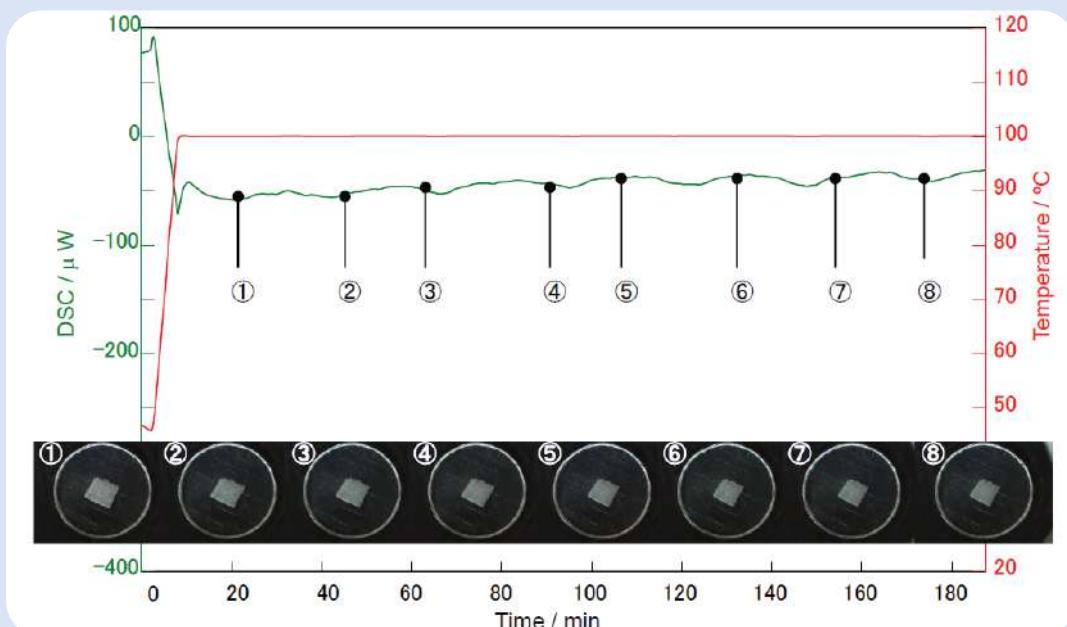
Images reveal changes in sample shape, size, color, and other properties. The images can be recorded and are automatically linked to the thermal data by timestamp.

HITACHI
Inspire the Next

Real-View DSC Measurement of Foamed Polystyrene

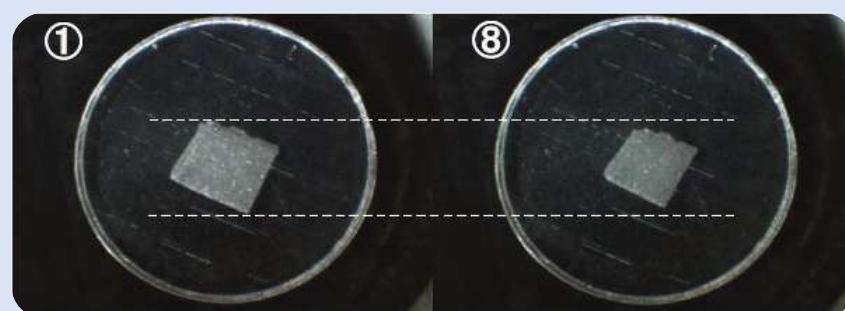
Foamed polystyrene is, as the name implies, a polystyrene material in the form of a hardened foam. It is lightweight, exhibits superior thermal insulating and elastic properties, is inexpensive, possesses good formability, and is used, among other things, as a cushioning and packing material, and as a thermal insulator.

Here, we show an isothermal analysis of the dimensional stability of foamed polystyrene using the Real-View DSC.



Polystyrene typically has a glass transition temperature near 100°C.

Above is an isothermal DSC curve for foamed polystyrene recorded at 100°C for 3 hours, together with images of the sample. Fluctuations are observed in the DSC curve but there are no signs of obvious changes. However, the Real-View images show that the sample is gradually contracting. The fluctuations are thought to reflect changes in the state of contact between the sample and the pan due to changes in sample shape.



100°C isothermal

- ▶ Left: after 20 min (① above)
- ▶ Right: after 180 min (⑧ above)

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Lyovapor™ L-250

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Key gains:

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Achieve an ice condenser capacity of 4 kg /24 h, with a total capacity of 5 kg.

② EcoStream™ Innovation

Innovative compressor design enables the condenser to reach temperatures as low as -85°C.

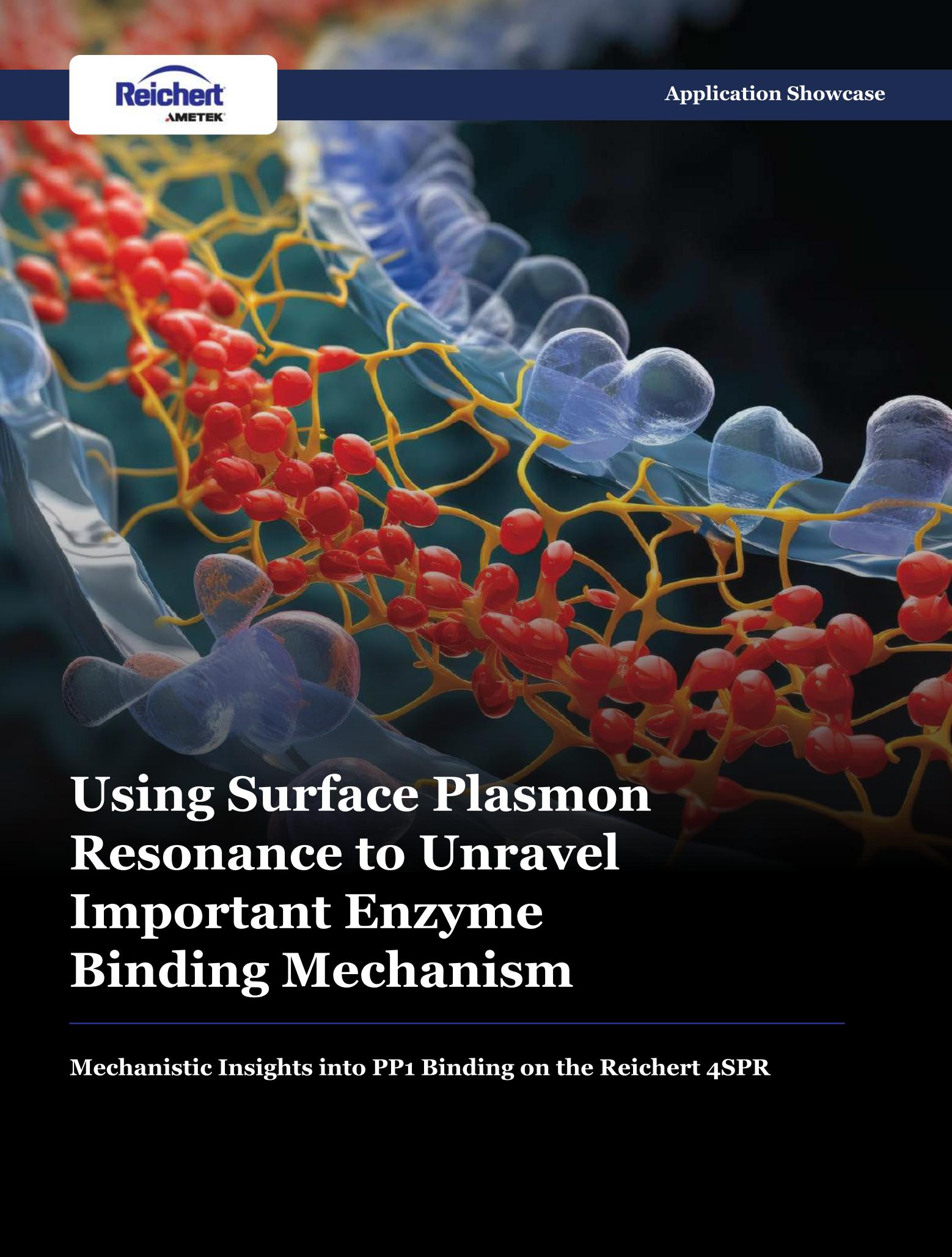
③ Embrace efficiency and elevate control

Featuring Infinite-Control™ technology that offers unprecedented levels of process control via display, software, and mobile app.

Certified sustainability by ACT label

Lyovapor™ L-250	
Lowest ice condenser temperature at 25° C	-85 °C
Dimension (WxDxH in mm)	503 x 645 x 510
Weight kg	67
Connection voltage	200 – 240 ± 10% VAC
Power consumption	1,300 – 1,800 VAC
Frequency	50 – 60 Hz
Environmental conditions	15 °C – 30 °C, max. relative humidity 80%
Minimum clearance on all Sides	30 cm
Noise level	< 68 dB
Minimum system vacuum (with vacuum pump / without samples)	0.03 mbar
Global Warming Potential (GWP)–Refrigerant	4,000
Leak rate	Max. 10.10 mbar x L / h





Using Surface Plasmon Resonance to Unravel Important Enzyme Binding Mechanism

Mechanistic Insights into PP1 Binding on the Reichert 4SPR

Researchers at the University of Arizona are studying Protein Phosphatase 1 (PP1), an enzyme involved in over 50% of all dephosphorylation reactions in the human body. In this current study, researchers sought to better understand how the known regulatory protein SDS22 interacts with PP1, including how it can act as both an activator and an inhibitor of PP1. Researchers used a number of techniques, including Surface Plasmon Resonance (SPR), to determine how different conformations of PP1 affect its binding to SDS22. They used their Reichert4SPR to help them better understand the role that metals play in the binding interaction.

Keywords or phrases:

Surface Plasmon Resonance (SPR), Protein Phosphatase 1 (PP1), SDS22 Regulatory Mechanism, Metal-Dependent Enzyme Binding, Reichert4SPR Analysis

INTRODUCTION

Protein Phosphatase 1 (PP1) is a Ser/Thr Phosphoprotein Phosphatase (PPP) that is abundant in many living organisms, including humans. PP1 is a single domain enzyme. It can form holoenzymes (combinations of enzymes with coenzymes that are biochemically active) with > 200 regulatory proteins. Previous research has shown that the active sites of all PPPs bind 2 metal ions (M1 and M2) and that the identity of the metals is dependent on the host¹. For instance, in mammals, the M2 metal is Fe²⁺ and the M1 metal is Zn²⁺. In bacteria, M1 and M2 are both Mn²⁺.

One of the regulatory proteins of PP1 is SDS22, which has long been known, but whose interaction with PP1 has not been fully understood. In this work, cellular, biophysical, and crystallographic studies were used to provide more information about the PP1:SDS22 interaction. Using crystallography, researchers were able to show that SDS22 binds to a form of PP1 that was not known previously and in

addition, they found out more about how it acts as an inhibitor. With SPR they were able to expand their knowledge of how the binding is carried out, including information on the kinetics of the interaction.

EXPERIMENTAL

BACKGROUND

Researchers were interested in better understanding the role of SDS22 in relation to PP1. They also wanted to determine what role the PP1 metals (M1 and M2) play in the PP1:SDS22 interaction.

CONDITIONS

- ▶ **Instrument:** Reichert4SPR
- ▶ **Sensor Chip:** Ni-NTA functionalized dextran (NiD50L; Xantec) Temperature: 25 °C
- ▶ **Targets:** PP1 variants
- ▶ **Analytes:** SDS22, Control NIPP1
- ▶ **Buffer:** 20 mM Tris pH 8.0, 500 mM NaCl, 0.5 mM TCEP, 0.05% Tween (with and without the addition of 1 mM MnCl₂ or 1 mM ZnCl₂)
- ▶ **Association:** 1.5 minutes
- ▶ **Dissociation:** 3 minutes
- ▶ **Flow Rate:** 50 µL/min
- ▶ **Regeneration:** 350 mM EDTA pH 8, 10 mM NaOH Recharge surface with 40 mM NiSO₄

RESULTS

Using multiple techniques, researchers determined that SDS22 binds exclusively to metal deficient PP1 (PP1 with M2 only).

To determine the mechanism, ie. whether SDS22 causes the loss of metal at the active site or binds PP1 after the metal has been lost, they used crystallography and surface plasmon resonance (SPR). Results indicated that SDS22 binds PP1 after the metal is lost¹.

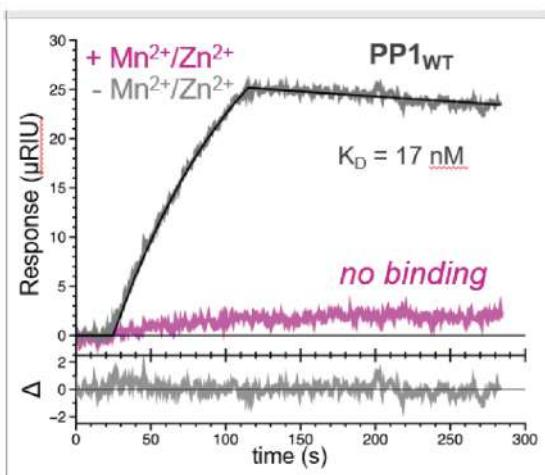


Figure 1. An example of the PP1:SDS22 interaction studied in metal containing and metal free buffers. SPR results show that SDS22 binds selectively to M1-free PP1.

Researchers also tried adding metal into the buffer at the dissociation step of the PP1:SDS22 binding interaction. They found that the dissociation was not affected (Figure 2), indicating that the addition of metal alone does not break up the interaction.

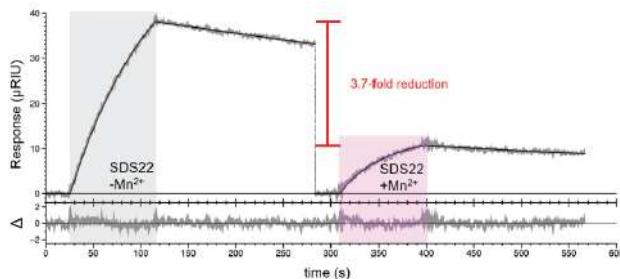


Figure 2. Mn²⁺ ion was added to the dissociation to see what effect it had. No change in the dissociation was seen.

SPR experimental results showed that the interaction between SDS22 and PP1 lacking M1 is tight, with a KD of 16.9 ± 5.1 nM. While this value is similar to that obtained for other PP1 regulators, the interaction is not typical in that the association (on) and dissociation (off) rates are both slower. Along with data obtained using other techniques, researchers were able to conclude that the slower off rate helps SDS22 stabilize and trap the inactive metal-deficient conformation of PP1¹.

There was an additional question whether Mn²⁺ and SDS22 compete for binding to PP1. It was confirmed they do with approximately a 3.7-fold reduction in SDS22 binding in buffer containing Mn²⁺ (Figure 3).

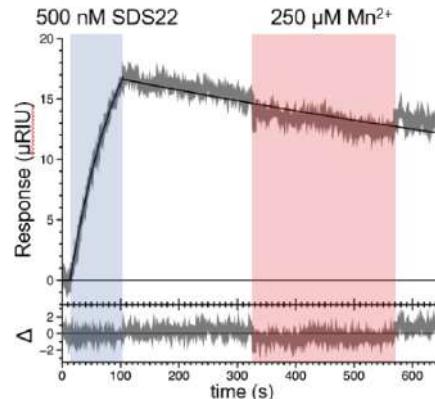


Figure 3. Sensorgrams without and with manganese ion in the injected sample. Manganese was excluded from the running buffer.

CONCLUSION

The key finding from this study is that researchers determined that SDS22 binds a previously unknown conformation of PP1 that contains a single metal (M2 only, not M1) at its active site. The PP1 with only one metal is inactive, so SDS22 is effectively trapping metal-deficient (inactive) PP1. The mechanism of the binding was also determined – i.e. data was obtained that confirms that SDS22 binding does not cause removal of the M1 metal from the PP1 active site, but instead, selectively binds PP1 that is lacking M1 metal at the active site (has M2 only).

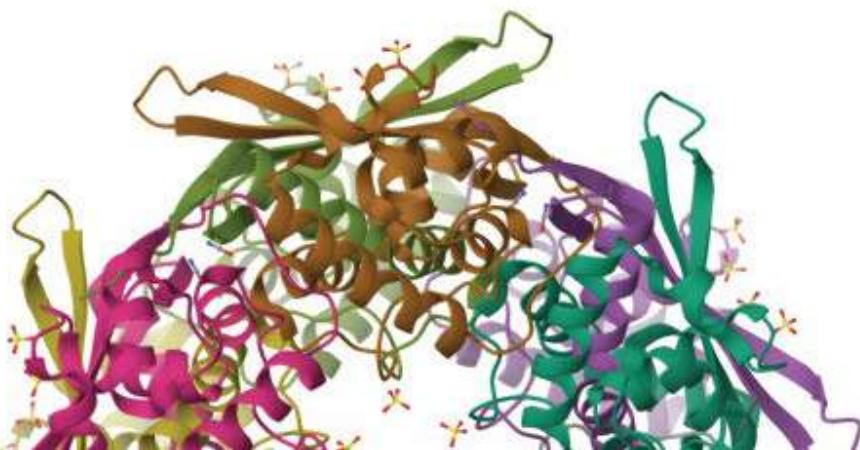
The dissociation of the complex is slow, indicating that SDS22 forms a tight, long-lived complex with PP1. In addition, when SDS22 dissociates, M1 metal is loaded into PP1. Hence, SDS22 provides a “pool” of inactive PP1 ready for holoenzyme formation when needed and it simultaneously prevents unregulated PP1 activity in the cell.

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Water Makes the Difference: Enhancing 2D Gel Electrophoresis Results with Ultrapure Water

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Sartorius Arium® Water Purification Systems

Are there differences in quality between stored and freshly prepared ultrapure water? This application presents the results for the use of ultrapure water in gel electrophoresis and provides recommendations for the production of ultrapure water.

Keywords or phrases:

Arium® Pro VF, ultrapure water, two-dimensional gel electrophoresis, 2D PAGE, protein separation, protein analysis, silver staining

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3. Sartorius Lab Instruments GmbH & Co. KG, 37079 Göttingen, Germany

INTRODUCTION

Two-dimensional gel electrophoresis, called 2D PAGE for short, was developed by Klose and O'Farrell independently of each other in 1975^{1, 2}. It is used to separate protein mixtures into individual proteins and today has become an indispensable method in protein analysis (proteomics).

This 2D procedure consists of two separation principles, known as dimensions (Figure. 1). While in the first dimension, ampholytes or immobilizing pH gradients (IPGs) are used to separate proteins according to their isoelectric points, in the subsequently second dimension, proteins are separated according to their molecular weights in SDS PAGE.

Afterwards, the separated proteins are visualized using different staining methods, (Coomassie blue, silver staining, etc.) and employed to perform subsequent downstream analysis, as needed.

The quality of the results of a 2D gel electrophoresis is affected by many factors: The type of electrophoresis system used, and the purity of the chemicals employed are decisive.

While the advantages of using horizontal high performance electrophoresis (HPE) over a conventional vertical system have already been shown³, this study examines the effect of water quality on silver staining.

Silver staining according to Blum et al.⁴ was used to visualize proteins after 2D gel electrophoresis. This method is characterized by a very high sensitivity and places high requirements on the purity of the chemicals and ultrapure water used, which is reflected in the intensity and number of spots. The influence of water quality on silver staining was studied.

Two different grades of water were tested for their suitability:

Ultrapure water produced by the Sartorius Arium® Pro VF water purification system and

Water from the same system that had been stored for one week in a glass bottle (Erlenmeyer flask made of Duran glass and supplied by Schott; covered by perforated parafilm) at room temperature in a light place (referred to in this paper as "flask water").

The ultrapure water used was prepared as follows:

The Arium® Pro VF system was used to generate ultrapure water for 2D gel electrophoresis. It removes impurities still present in pretreated tap water.

Production of ultrapure water requires continuous recirculation and a constant water flow, which is achieved by a pump system with pressure control.

The conductivity of the water is measured at the feed water inlet and in the product water (ultrapure water dispensed directly from the water outlet).

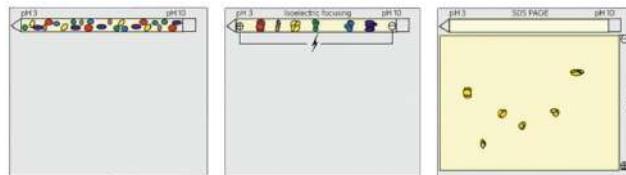


Figure 1: Principle of 2D gel electrophoresis: Isoelectric focusing (IEF) is performed in the first dimension to separate proteins according to their isoelectric points (center image). In the second dimension, proteins are separated in SDS electrophoresis (for SDS PAGE, see image on the right) according to their molecular weights.

Used in the studies described in this paper, the Arium® Pro VF is a predecessor model with identical technical specifications for production of ultrapure water as the redesigned system shown above and works with two different cartridges.

These are filled with a special active carbon absorber and mixed bed ion exchange resins in order to deliver ultrapure water with a low TOC content (total organic carbon).

In addition, a UV lamp is integrated into the system, which has oxidizing and germicidal effects at wavelengths of 185 nm and 254 nm.

Furthermore, the Arium® Pro VF system has a built-in ultrafilter module used as a crossflow filter. The ultrafilter membrane it employs retains colloids, microorganisms, endotoxins, RNA, DNA and RNases, which is essential for the 2D gel electrophoresis runs performed and for subsequent silver staining. A 0.2 µm final filter installed on the water outlet serves to remove particles and bacteria from the ultrapure water produced. The process of water purification

MATERIALS AND METHODS

The materials used (chemicals and equipment) were supplied by Serva Electrophoresis GmbH, Heidelberg, Germany, if not otherwise specified.

Escherichia coli (E.coli) extract: E.coli extract was prepared by two freeze-thaw cycles of lyophilized E. coli cells (ATCC 11303, Sigma-Aldrich Chemie, Munich, Germany) in 9 M

urea, 65 mM dithiothreitol (DTT), 4% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propansulfonate (CHAPS), 0.8% (v/v) Servalyt 3-10.

2D gel electrophoresis and silver staining: Isoelectric focusing (first dimension) was performed with Serva IPG Bluestrips, with a length of 11 cm, at a pH 4 – 7 in a Hoefer IEF 100 IPG focusing chamber (Figure 2a). For this purpose, the IPG strips were rehydrated overnight with 100 µg E.coli extract in 190 µL rehydration solution (8 M urea/0.5% CHAPS/0.2% DTT/0.5% Servalyt 3-10(v/v)) per strip.

Afterwards, focusing was performed using the following program: Current per IPG strip: 75 µA, 250 V 1 h; 500 V 1 h; 5,000 V 30 min and 5,000 V to 27,000 Vh.

Before SDS PAGE (second dimension) was carried out, each of the focused IPG strips was equilibrated for 15 minutes in 30% glycerol, 6 M urea, 2% SDS, 50 mM Tris with a pH of 8.8, 1% DTT and 30% glycerol, 6 M urea, 2% SDS, 50 mM Tris with a pH of 8.8, 2.5% Iodacetamide.

SDS PAGE was run on a 2D HPE large format gel, 12.5%, in an HPE Blue Horizon chamber. IPG strips were placed on a gel.

Separation of the proteins was carried out under the following running conditions:

$V = 100 \text{ V}$, $I = 7 \text{ mA}$, $P = 1 \text{ W}$, $t = 30 \text{ min}$;

$V = 200 \text{ V}$, $I = 13 \text{ mA}$, $P = 3 \text{ W}$, $t = 30 \text{ min}$;

$V = 300 \text{ V}$, $I = 20 \text{ mA}$, $P = 5 \text{ W}$, $t = 10 \text{ min}$;

$V = 1,500 \text{ V}$, $I = 40 \text{ mA}$, $P = 40 \text{ W}$, $t = 3 \text{ h } 20 \text{ min}$

Following gel electrophoresis, the gel was halved. To visualize the protein spots, silver staining was performed according to Blum et al.⁴. All solutions needed for staining were prepared either using ultrapure water dispensed from the Arium® Pro VF system or with “flask water” to compare the effect of water quality on staining. The gels were fixed for 30 minutes in 40% ethanol and 10% acetic acid and washed three times with 30% ethanol for 10 minutes each time.

Afterwards, one-minute pretreatment with a sodium thiosulfate solution, staining with a silver nitrate solution and development using a sodium carbonate | formaldehyde solution were performed. Silver staining (Figure 2) was stopped using a 1% glycine solution. The gels stained in this manner were evaluated using Delta 2D software supplied by Decodon (Figure 3).

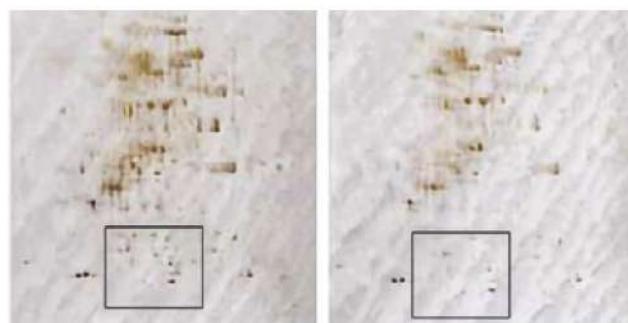


Figure 2. Silver staining of the gels: left, Sartorius Arium® Pro VF water; right, “flask water.” The marked area shows the regions whose densitograms were compared (Figure 3).



Figure 3: Three-dimensional densitograms of the areas marked in Figure 2. Left: fresh Sartorius Arium® Pro VF water; right: “flask water”.

RESULTS

“Flask water” (Figure 2, right) used in silver staining according to Blum resulted in significantly poorer spot intensities than did the freshly prepared ultrapure water (Figure 2, left), which is indicated by larger spot areas and weaker spot volumes. The number of spots of 348 remained nearly unchanged.

As an example, the regions marked in Figure 2 were compared with respect to spot areas and spot volumes.

Thirty-six spots (Figure 3) were more closely examined.

The spot ratio areas and volumes are depicted graphically in Figure 4.

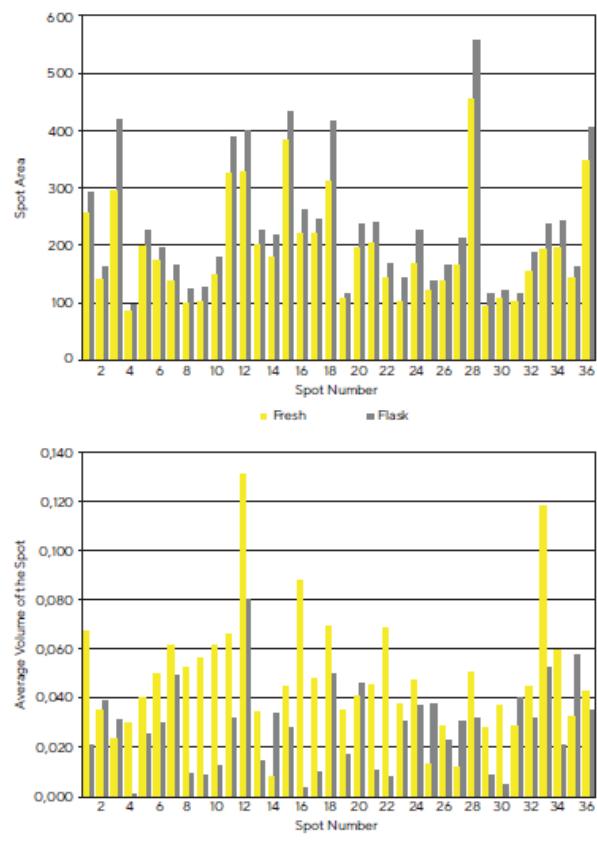


Figure 4: Graphic representation of area and volume of the spots marked in Figure 3.

DISCUSSION

The effects that the use of stored ultrapure water can have are shown by the results of silver staining: the sharpness of the spots correlates with the purity of the water.

Poorer water quality results in the decreased quality of silver-stained spots, which are diffuse, have a larger area and a less intensive stain. This signifies a lower protein quantity per area and is disadvantageous for quantification and subsequent analyses, e.g., if spots need to be excised using a spot picker to further process these gel plugs for subsequent mass spectrometry.

Poorer silver staining quality lets us conclude that the also

be. Afterwards, one-minute pretreatment with a sodium thiosulfate solution, staining with a silver nitrate solution and development using a sodium carbonate + formaldehyde solution were performed. Silver staining (Figure 2) was stopped using a 1% glycine solution. The gels stained in this manner were evaluated using Delta 2D software supplied by Decodon (Figure 3).

ACKNOWLEDGEMENTS

At this point, the authors would like to thank Steffen Sander of Sartorius Lab Instruments for his practical support and Dr. Doris Fernholz-Horstmann of Serva Electrophoresis for reviewing the manuscript and for her constructive comments in discussing this topic.

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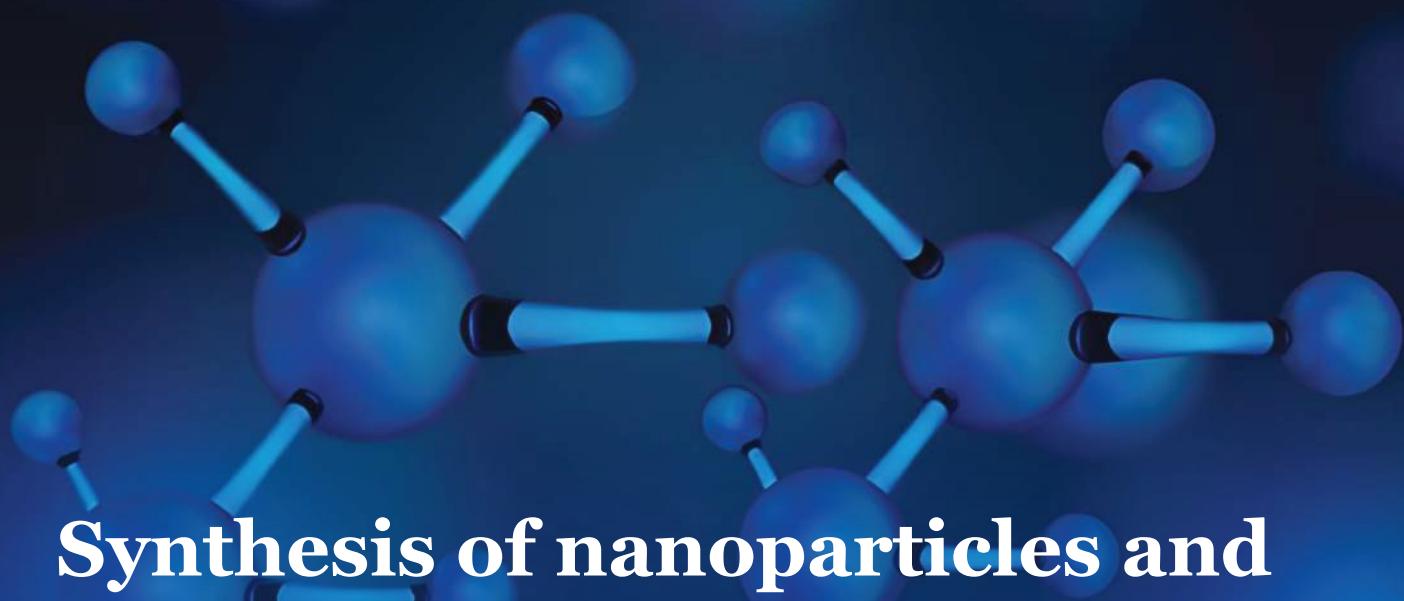


Food & beverage quality testing



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Synthesis of nanoparticles and their efficient use in the H-Cube® and H-Cube Pro™ flow reactors for reductions and H-D exchange reactions

**ThalesNano Solutions in Nanoparticle Catalysis for
Flow Chemistry**

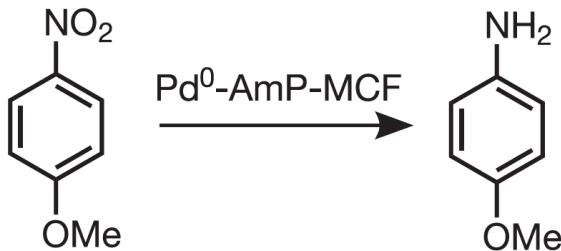
This application demonstrates the results of newly developed Pd, Ni nanocomposites, and immobilized Ir complex catalysts for a wide range of chemical applications.

Keywords or phrases:

ThalesNano, nanoparticle, catalysts, flow chemistry, hydrogenation, H-D exchange

Pd NANOPARTICLES FOR NITRO REDUCTION

Verho et al.¹ detailed how palladium nanoparticles supported on amino-functionalized mesocellular foam can be used under mild conditions for the selective hydrogenation of aromatic and heterocyclic nitro compounds to amines, which are intermediates for the production of various dyes, pharmaceuticals, pigments, and polymers. The catalyst (Pd⁰-AmP-MCF) was tested for both batch and continuous methods. In the batch reference 1.60 mmol starting material (p-nitroanisole) was treated with atmospheric pressure hydrogen in the presence of 0.5 mol% catalyst in 2 mL EtOAc at room temperature. After 1 hr of reaction time the corresponding amine could be collected in >99% yield. Using the H-Cube[®] continuous flow reactor, 70 mg of this metal nanoparticle was filled into the catalyst cartridge (CatCart[®]), and the mixture of starting material and in-situ generated high pressure hydrogen was reacted inside the CatCart[®] at atmospheric pressure. 2.50 g p-nitroanisole in EtOAc was continuously pumped though the catalyst bed at 1.5 mL/min and 40 °C reaction temperature resulting in 0.90 g (95% yield) final product after 110 min with <1 ppm Pd content. The experiments showed that the Pd nanoparticle, which is a green alternative to the bulk metal catalyst, is active both under batch and flow conditions.



NICKEL NITRIDE NANOPARTICLES FOR REDUCTIONS

At the Max-Planck-Institute for Colloids and Interfaces there is an extensive research towards different metal nitride nanoparticles. One of their publications is about nickel nitride², which was mainly investigated for lithium batteries. There are different approaches for the synthesis of these nitrides. One of them is the “urea glass route”, which is a simple, scalable and versatile method: the first step is the formation of metal-urea gel followed by thermal decomposition under inert atmosphere. The used urea also forms the carbonous matrix (size: around 25 nm), which directs the formation of nanoparticles and prevents their aggregation. Depending on the used temperature, the reaction can result in a Ni³N@carbon hybrid composite at 350 °C, and Ni³C at a higher temperature.

The produced catalysts were then filled into CatCarts[®] and used in the H-Cube Pro[™] system for nitro group, double bond, and nitrile group reductions. Results of the experiments are summarized in Table 1 using 4.33 mmol Ni³N ~630 mg, or ~305 mg Ni, and 0.05 M starting material in EtOH. Both nanocomposites show high activity at low temperatures for the different functional groups. It is notable that while they represent similar activity for nitro reduction, the Ni³N nanocomposite was not able to reduce cyclohexene.

However, an interesting phenomenon occurred for the highly temperature sensitive nitrile reduction, when the use of Ni³N catalyst allowed the production of dibenzylimine.

Substrate	Catalyst	Flow rate (mL/min)	p (bar)	T (°C)	Conversion (%)	Product
Nitrobenzene	Ni	0.5	25	50	>99	aniline
Nitrobenzene	Ni3N	0.5	25	75	>99	
p-nitroacetophenone	Ni	0.3	25	75	>99	4-aminoacetophenone
p-nitroacetophenone	Ni3N	0.3	25	75	>99	
Cyclohexene	Ni	0.3	12	25	>99	Cyclohexane
Cyclohexene	Ni3N	0.5	12	75	>10	
Benzonitrile	Ni	0.5	25	55	>99	>99% dibenzylamine
Benzonitrile	Ni3N	0.3	25	75	>99	22% benzylamine, 69% dibenzylimine, 8% dibenzylamine
Benzonitrile	Ni3N	0.5	25	135	>99	88% benzylamine, 9% dibenzylimine, 4% dibenzylamine

Table 1. Reaction parameters and results of hydrogenation using Ni and Ni3N nanocomposites

TITANIUM NITRIDE – NICKEL NANOCOMPOSITE FOR THE HYDROGENOLYSIS OF ARYL ETHERS

As described by Molinari et al., titanium nitride was also synthesized similarly to the nickel nitride, and the TiN was used as support to enhance the catalytic activity of Ni³⁺. TiN is a biocompatible material with thermal stability and acid resistance. TiN nanoparticles were synthesized via the previously described urea route. Calcination was performed at 750 °C. Addition of Ni was performed by impregnation of TiN with Ni acetate tetrahydrate in EtOH, followed by calcination. The TiN–Ni nanocomposite is composed of spherical intergrown core–shell nanoparticles of ~10 nm in diameter. The produced catalysts (TiN and TiN–Ni) were then tested for the hydrogenolysis of aryl ethers, together with control experiments using Ni. The selected compounds are the most common aryl ethers found in lignin.

All catalytic reactions were performed using H-Cube ProTM. Starting with a control experiment of hydrogenation of nitrobenzene with Ni, TiN and TiN–Ni (500 °C), TiN did not show any catalyst activity in a temperature range of 25–100 °C. Ni particles exhibited a moderate activity at 100 °C, while 100% conversion was achieved with the TiN–Ni nanocomposite, even at 25 °C. Finally different aryl ethers

were hydrogenated, as shown in Table 2, indicating a significant activity increase for the TiN–Ni catalyst for all the three compounds.

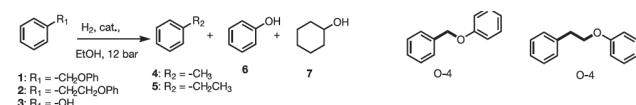
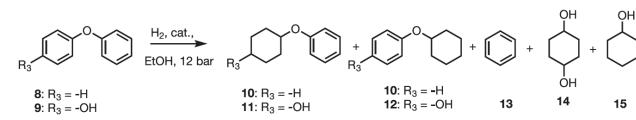


Figure 2: Hydrogenolysis of ethyl ethers

Entry	Catalyst	Substrate	T (°C)	Conversion (%)	4	5	6	7	Product (mmol h ⁻¹ g ⁻¹)
1 ^a	Ni3N	1	125	18	51	–	49	–	0.225
2 ^a	TiN–Ni	1	125	>99	54	–	46	–	1.013
3 ^a	Ni	2	150	0	–	–	–	–	–
4 ^a	TiN–Ni	2	150	>99	–	54	–	46	0.608
5 ^a	TiN–Ni	3	150	55	–	–	–	100	0.62

Table 2. Reaction parameters and results of ethyl ether hydrogenolysis. a: 0.05 M, 0.5 mL/min; b: 0.3 mL/min. Selectivity and conversion were determined by GC-MS



Furthermore, the research was extended to the above displayed 4-O-5 bond, which is one of the strongest ether bonds in lignin. Its hydrogenolysis was modelled using diphenyl ether. The hydrogenolysis was carried out with Ni,

TiN and TiN–Ni at 150 °C. Neither Ni nor TiN were capable of efficiently performing the reaction, while TiN–Ni showed 99% and 65% for the diphenyl ether and its hydroxyl derivative respectively when applying a 0.3 mL/min flow rate to the 0.025 M substrates.

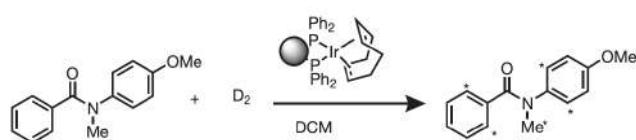
During the hydrogenation of diphenylether 46% of 13 and 49% of 15 were formed, whereas the presence of the –OH group changed the product distribution to 49% of 13, 25% of 14, and only 4% of 15. Qualitative evaluation confirmed that the catalyst remained active even for a ~40 hr reaction time.

H-D EXCHANGE WITH IRIDIUM COMPLEX

The members of the H-Cube Series generate hydrogen via electrolysis of water. Consequently, when heavy water is filled into the reactor the corresponding deuterium gas is generated and introduced into the reaction zone at high pressure resulting in deuterated compounds after the reaction. Deuterium labelled materials play a key role in drug discovery for studying drug metabolism.

Deuterated compounds are ideal for stable isotopically labelled internal standards for LC-MS/MS techniques, but due to the high cost of deuterium gas, and the associated safety issues, their use is not widely supported. In the application from Habraken et al.

the first use of Ir(I)-catalyzed C–H deuteration in a continuous flow manner is detailed with a comparative study using batch, continuous stirred tank reactor (CSTR), and two types of prepacked reactors (microreactor and H-Cube Pro™)⁴.



Entry	Reactor type	Gas flow rate (mL/min)	Liquid flow rate (mL/min)	Conversion (%)	M + 1 (%)	M + 2 (%)	M + 3 (%)
1 ¹	CSTR	0.02	0.08	12	8.1	3.6	–
2 ¹	CSTR	0.02	0.025	16	9.8	4.8	–
3 ¹	CSTR	0.08	0.085	2.8	2.8	–	–
4 ²	Microreactor	0.04	0.01	54	4.9	16.2	12.1
5 ²	H-Cube Pro™	40 bar	0.3	56	14.3	38.4	2.8
6 ^{2,3}	H-Cube Pro™	40 bar	0.3	64	14	43.4	4.5

Table 3. Reaction parameters and results of deuteration reaction; 1: 1 mg/mL SM, 10 mg catalyst, RT, yields and conversion (determined by LC-MS) are the average of 3 independent points; 2: 1 mg/mL SM, 100 mg catalyst, RT, yields and conversion (determined by LC-MS) are the average of 5 independent points; 3: the liquid stream is the one exiting entry 5 which is redirected over the catalyst bed.

As it can be observed from Table 3, the CSTR with its 0.7 mL mixing chamber and deuterium feeding through a mass flow controller, could result only in a maximum 16% conversion with a maximum deuterium incorporation of M⁺².

With the use of prepacked microreactor 54% conversion was achieved, and the product mixture contained deuterium incorporation up to M⁺⁷ (not displayed in Table 3).

Using the H-Cube Pro reactor there was no need for a mass flow controller and deuterium tank, because the deuterium was produced from deuterated water, and the reaction pressure could be set to 40 bar. In this way 64% conversion was achieved as a maximum.

CONCLUSION

The above examples demonstrate the usefulness of flow chemistry in the testing of different nanoparticles in various hydrogenation reactions and for H-D exchange reaction.

The combination of using nanoparticles and flow chemistry further increases the sustainability of the processes and makes the involvement of expensive catalysts and reagents or dangerous methods unnecessary.

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H-Cube® Pro



Flow reactor for advanced hydrogenation

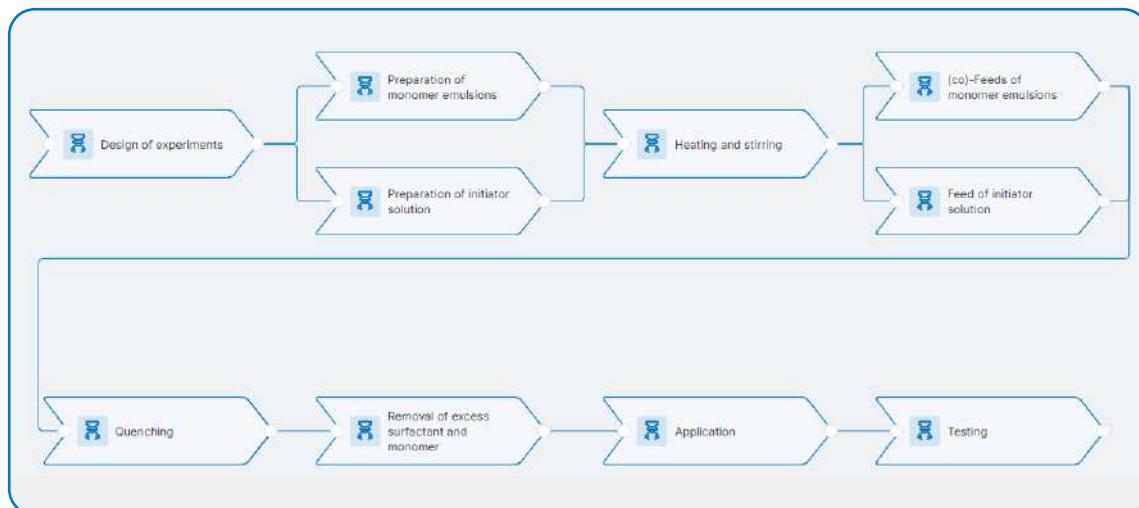
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EXAMPLE WORKFLOW



POWERFUL ADVANTAGES

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- ▶ Powerful mixing for viscosities up to 80 Pa.s at 300 rpm and 30 Pa.s at 900 rpm with an anchor stirrer.
- ▶ Flexible and precise continuous feeds.
- ▶ Multiple continuous liquid, liquefied gas and / or gas feeds per reactor and additional unlimited overhead access.
- ▶ Independent, precise temperature and stirring control in each reactor (e.g. 130°C temperature difference between adjacent reactors, with active cooling >130°C).
- ▶ Internal and / or jacket temperature control.
- ▶ Pressure up to 100 bar with corresponding safety installations.
- ▶ Parallel high-performance calorimetry and viscosity data.
- ▶ PAT interfaces to various in-situ probes, for example: pH, UV-VIS, IR, Raman, PSD, calorimetry.
- ▶ Measurement of gas consumption.
- ▶ Dean Stark water trap.
- ▶ Distillation bridge.
- ▶ Vacuum for different purposes: inertization, crystallization, etc.
- ▶ Extraction, filtration, evaporation, crystallization, distillation.
- ▶ Ventilated hood for safety and conditioned (sensitive chemicals, low-temperature) operation.
- ▶ AUTOSUITE SOFTWARE – drag & drop experimentation with easy interface (e.g. python custom device) to e.g. LIMS, ELN, data analysis, AI / ML closed loop.

CASE STUDY

AUTOMATION AT WORK: ENABLING BREAKTHROUGHS IN POLYMER RECYCLING WITH CHEMSPEED

The Challenge

Recycling PET into valuable monomers is a cornerstone of circular plastics. Yet researchers face a long-standing trade-off: homogeneous catalysts deliver excellent efficiency but are hard to recover, while heterogeneous systems simplify handling but often sacrifice performance. Developing catalysts that combine both benefits requires rapid synthesis, precise control, and systematic testing — something traditional lab workflows struggle to deliver.

The Chemspeed Advantage

To overcome this bottleneck, researchers turned to Chemspeed's automation platforms.

- ▶ The ASW 2000 automated parallel synthesizer enabled high-throughput RAFT polymerizations, producing a library of thermo-responsive polymer catalysts with consistent quality.
- ▶ The FORMAX platform allowed parallel PET glycolysis

- ▶ reactions, with each reactor independently controlled for temperature, stirring, and reagent dosing.
- ▶ Integrated gravimetric dispensing and multi-needle liquid handling ensured reproducibility while minimizing operator time.

The Breakthrough

- ▶ Achieved >91% PET depolymerization conversion with >90% selectivity for the desired monomer (BHET).
- ▶ Demonstrated programmable, thermo-responsive polymer catalysts capable of switching between homogeneous and heterogeneous modes.
- ▶ Reduced manual setup time and experimental variability, freeing researchers to focus on scientific discovery instead of repetitive tasks.

The Impact

This case study shows how Chemspeed's automation backbone accelerates sustainable innovation. By merging parallel synthesis with automated application testing, researchers developed catalysts that push the boundaries of recycling technology—pointing toward a future of fully circular materials and processes.

With Chemspeed, the path from idea to impact is not just faster—it's smarter.



FLEX AUTOPLANT

Fully Automated Solution for Automate process R&D and Preparative Synthesis



FiveEasy™ Plus pH meter FP20-Bio-Kit

METTLER TOLEDO



Precise pH control is essential in all laboratories. With the FiveEasy™ FP20-Bio Kit, you have an easy-to-use instrument that makes pH measurement easy, convenient, and reliable. With intuitive use, reliable and steady performance, and a simple display, the FP20-Bio Kit keeps your focus on results, not on tedious setup steps.

Perfect for everyday pH use in college, industrial, and quality control laboratories, this convenient benchtop meter offers high-quality measurements without a break in the bank. From rapid checks to daily series of samples, the FP20-Bio Kit is the ideal partner for trouble-free, dependable pH analysis.

Intuitive Operation—Measurement Made Easy

Featuring a large, well-structured display for easy viewing, the instruments' intuitive button arrangement and simple menu ensure measurements can be performed in just a few clicks



Compact Design—Save Space

Thanks to its compact design, little bench space is needed for the Five meters. The sensor holder is integrated into the housing so that the sensor can be easily removed and stored on the side of the meter after use.



Fast Connectivity—Simple and Secure Data Transfer

The FiveEasy Plus FP20 Bio-Kit meter allows data to be exported either directly to a printer or a PC for further processing using its RS232 or USB port.



Parameter	Value
Channel	Single-channel
Sensor	LE410
pH measuring range	-2 – 16
pH resolution	0.01 / 0.1
pH accuracy (±)	0.01
mV measuring range	0 – 0
mV resolution	1
mV accuracy (±)	1
Temperature Range	-5 °C – 105 °C
Temperature resolution	0.1 °C
Temperature accuracy (±)	0.3 °C
Ease of Use	Calibration support

Applications

Optimally designed for work on bench surfaces in a wide range of industries, including laboratories in academia, food and beverage, environment, and agriculture.



Food & Beverage



Environment



Agriculture



Industry



Academia

FiveEasy Plus pH meter FP20-Bio-Kit

For Effortless Routine Measurements

Built-in Strengths

- » pH range: -2.00 to 16.00
- » Accuracy: ± 0.01 pH
- » Up to 5-point calibration with predefined buffers
- » Automatic & manual temperature compensation
- » Direct data export via USB/RS232 – no manual errors
- » A large, well-structured display, intuitive button arrangement and a simple menu
- » Compact design with side-mounted sensor holder saves bench space.



Fast routine pH & mV checks – save time everyday

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pH Made Easy: Practical Steps for Accuracy

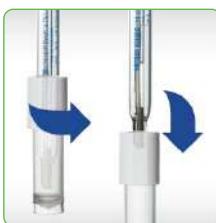
The accuracy of a pH measurement is influenced by different factors, such as the accuracy of the buffers used for calibration, whether or not temperature compensation is used, and if the right electrode is used for a particular application, to mention just a few. When great care is taken with the measurements, an accuracy of ± 0.05 pH units could be achieved.

This step-by-step guide assumes that a combination pH electrode (measuring and reference electrodes in one) is used. If separate measuring and reference pH electrodes are used, ensure that you always put the electrodes in the same solution during the measurements. Also ensure that both electrodes are connected to the pH meter.

ELECTRODE PREPARATION



- 1a. Connect the pH electrode and temperature probe to the pH meter.



- 1b. Remove the wetting cap from the pH electrode.



- 1c. Check the tip of the electrode for air bubbles. If found, shake the electrode like a thermometer.



1d. For refillable electrodes only: open the refilling hole before use.



1e. For refillable electrodes only: check the electrolyte level (artificially coloured green for illustration purposes) and refill if necessary.



1f. Rinse the electrode with distilled or deionized water.

The electrode is ready to perform a calibration or a measurement.

2. ELECTRODE CALIBRATION



2a. Select the correct buffer group or buffer values for the calibration in the meter's settings.



2b. Pour enough pH calibration buffer into a measuring beaker and dip the pH electrode into the first buffer.



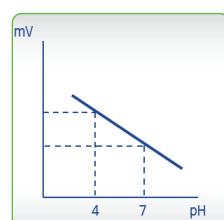
2c. Press the calibration button on the pH meter and wait until the measurement is stable.



2d. Take the electrode out of the solution and rinse it with distilled or deionized water.



2e. Repeat steps 2– 4 for each calibration buffer used.



2f. Review the calibration results on the meter and save them if they are acceptable.

3. MEASUREMENT



3a. Pour enough sample solution into a measuring beaker and dip the pH electrode into the sample.



3b. Press the measurement button on the pH meter and wait until a stable endpoint has been reached.



3c. Take the electrode out of the solution and rinse it with distilled or deionized water.

In case rinsing with distilled water in step 3 is not sufficient, perform the following procedure:

Rinse the electrode with ethanol or acetone until all contamination is removed.

Rinse again with deionized water.

Condition the electrode by dipping it in an electrolyte solution for several minutes.

- Calibrate after every cleaning step with an organic solvent.



3d. For additional samples repeat steps 1– 3 until all samples have been measured.

4. ELECTRODE STORAGE

For short term storage



Place the electrode in a beaker with reference electrolyte solution or pH buffers 7.00 or 4.01.

For long term storage



Close the refilling hole.



Fill the wetting cap with the appropriate electrolyte.



Put the wetting cap on the electrode. Make sure that the whole membrane is covered with electrolyte solution.



Store the electrode vertically, membrane down.

GOOD pH MEASUREMENT PRACTICES

Measuring pH is generally considered an easy, fast, and simple process, but so many things can go wrong. Different measurement and maintenance practices are essential to ensure the reliability and quality of pH measurements:

- Select the correct electrode for your sample:** pH electrodes play a very important role in performing correct pH value determinations, since they are responsible for the actual pH measurement. Based on your application, select the most suitable electrode.
- Use the correct calibration buffers:** The buffers used for calibration must be selected according to the sample's pH. For instance, if a sample is expected to have a pH of 7.45, the calibration must include pH buffers 7.00 and 9.21 (or similar). Use a minimum of two fresh buffers for calibration.

4. Check your calibration results: The electrode calibration provides valuable data on the electrode condition. The slope of the calibration curve should lie between 95% and 102% of the theoretical value. Another measure of a good calibration is the offset at the zero point (0 mV at pH 7), which should remain relatively stable and should not exceed ± 30 mV.

5. Stir the sample: When measuring pH, gently stir the sample to ensure that it is homogeneous. It is important that calibration and measurement are done under the same conditions. Do not use the electrode to stir.

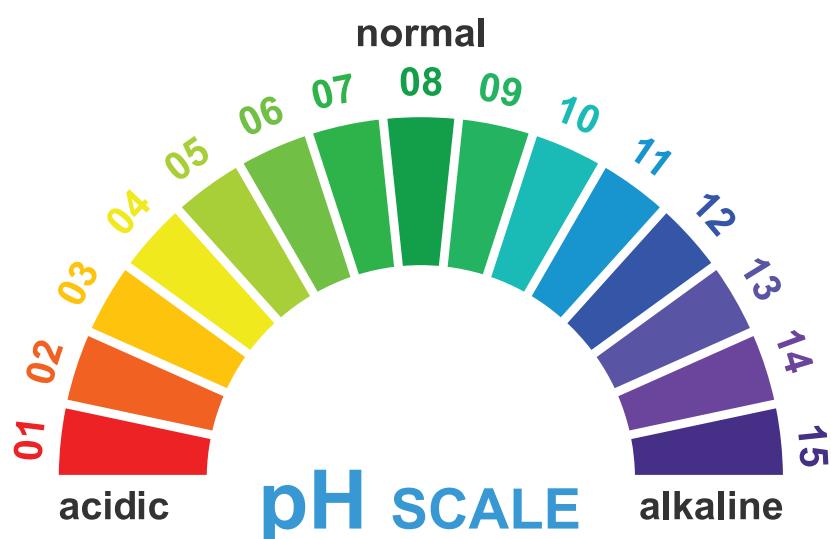
6. Calibrate your pH electrode daily: Regular calibrations will lead to more accurate results. Some applications might require a calibration before every measurement, but in general it is enough to calibrate every 24 hours.

7. Measure the temperature of the sample Every sample has a different pH behaviour depending on the temperature; therefore, the temperature should be measured. The temperature probe is either integrated in the pH electrode or an external one can be used. If the electrode and the sample have different temperatures, give the system enough time to equilibrate. To compare the pH of different samples, they should always be measured at the same temperature.

8. Use fresh buffers for calibration Check the expiry date of the calibration buffers before use. Never calibrate the sensor directly in the bottle. Do not re-use calibration buffers and never pour them back in the bottle. Close the bottles immediately after use and store them at room temperature.

9. Keep the electrode cleaned Using a dirty electrode is one of the typical sources of error in pH measurement. Make sure that the electrode is always clean and well maintained.

10. Store the electrode in a proper solution Electrodes should always be stored in aqueous and ion-rich solutions. The electrode should never be stored dry or in distilled water as this will affect the pH-sensitive glass membrane and thus shorten the lifetime of the electrode. If not sure which storage solution to use, check the electrode's manual.



CONSISTENT pH ACCURACY COMES DOWN TO PREPARATION, MEASUREMENT AND CARE.

GLOSSARY

pH	Chemical property of an aqueous solution, which indicates its degree of acidity (pH values from 0 to 7) or alkalinity (pH values from 7 to 14). A pH value of 7 is considered neutral.
pH meter	Potentiometer that measures the voltage difference between the glass electrode and the reference electrode and calculates the pH value.
pH electrode (also known as pH sensor or probe)	pH electrodes are responsible for the actual pH measurement, and it is therefore crucial to use the right pH electrode for each application. In general, assumed to be a combined electrode (a glass electrode and a reference electrode in one), which is immersed in the sample.
Measuring or glass electrode	It is the part that actually senses the pH of the solution. It consists of a glass shaft with a thin glass sensitive membrane at the tip. It can be combined with the reference electrode in just one electrode (combined pH electrode).
Reference electrode	It is the part that provides a defined stable reference potential for the pH sensor potential to be set against. It can be combined with the measuring electrode in just one electrode (combined pH electrode).
Junction (also known as diaphragm)	It is the connection between the reference electrolyte and the sample. It can have several different shapes and properties, depending on the application the electrode is aimed for.
Reference electrolyte	Solution that defines the potential of the reference electrode system. Which type of reference electrolyte is used in an electrode strongly depends on the reference system and the application.
Glass membrane	The glass membrane is the pH sensing part of the sensor. Its shape and glass composition are optimized to ensure best results in specific applications.
Buffer solutions or calibration standards	Standard solutions of known pH used to calibrate the pH electrodes and to check their performance.
Calibration	pH measurements in calibration standards, which establish the difference between what should be measured and what a pH electrode actually measures. It is followed by an adjustment to compensate for any
Sample	Solution to be measured. It needs to be an aqueous solution or to contain enough water for the pH measurement to be possible.

REFERENCE

A guide to pH measurement, 51300047

www.mt.com/Library

METTLER TOLEDO



Isolation Of Oligonucleotides by Scalable IEX-Centrifugal Partition Chromatography

There is a growing need for simple, rapid and if necessary, easily scalable chromatographic purification of oligonucleotides. The use of ion-exchange (IEX) chromatographic techniques has increasing potential, moreover, liquid-liquid chromatography can be used to develop easily scalable and cost-effective solutions without the need for expensive ion-exchange resins. In this study, we demonstrate the potential of our centrifugal partition chromatographic solutions for the separation of oligonucleotides.

THE TECHNOLOGY

Centrifugal partition chromatography (CPC) is a preparative liquid- liquid chromatographic technique, where both the stationary and the mobile phase are liquids and resolution is governed by the partitioning of solutes between these two immiscible liquid phases. In practice, one of the two phases

Keywords or phrases:

Oligonucleotide purification, Ion-Exchange Chromatography (IEX), Centrifugal Partition Chromatography (CPC), scalable separation, high-purity yield, RotaChrom CPC Modeler



CPC Modeler

is immobilized by a strong centrifugal force inside a rotor, while the other one, which contains the sample to be purified, is pumped through the rotor. CPC is a very loadable system depending on the amount of exchanger and displacer salts, however for smaller sample quantities (100–200 mg) the concentration of additives can be reduced, thus further reducing costs. A challenge in oligonucleotide separation is the presence of endotoxins, which are difficult to remove. The solvent system used in CPC can separate endotoxins, insomuch as n-butanol/water extraction of endotoxins is a potential solution.

CASE STUDY

The starting material was a 20-mer single-stranded unmodified oligonucleotide (dT), with 88.5% purity (Figure 1).

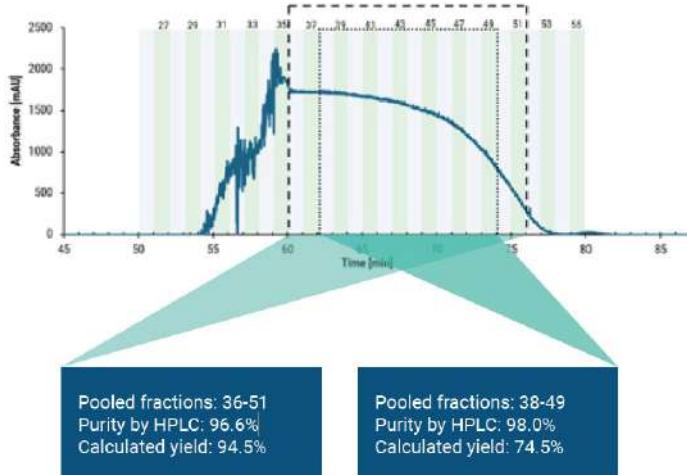


Figure 1: AEX-HPLC analytical chromatogram of the crude sample.

There is a growing need for simple, rapid, and scalable chromatographic purification methods for oligonucleotides. While ion-exchange (IEX) chromatography has growing potential, liquid-liquid techniques such as centrifugal partition chromatography (CPC) offer cost-effective alternatives without the need for expensive ion-exchange resins.

In this study, we demonstrate the effectiveness of our CPC platform for oligonucleotide purification.

Our two-phase solvent system consisted of ethyl acetate/n-butanol/nuclease-free water in a defined composition. The cationic compound (used as the exchanger molecule) was dissolved in the stationary phase to retain the oligonucleotides, while sodium hydroxide and sodium chloride in the mobile phase facilitated elution. The 600 mg sample was dissolved in 10 ml of mobile phase and injected via an injection loop at a flow rate of 10 ml/min. Method parameters are shown in Table 1.

Parameter	Specification
Instrument	CPC Modeler
Rotor volume	225 ml
Rotation speed	2200 rpm
Injected sample	600 mg / 10 ml SS lower phase via loop
Flow rate	10 ml/min
Mode	Descending (85 min)
Fraction collection	0.5 min (5 ml)
Stationary phase	SS upper phase, 60 mM cationic exchanger
Mobile phase	SS lower phase, 10 mM NaOH, NaCl

Table 1: Measurement parameters on the CPC Modeler.

The experiment was performed on RotaChrom's Benchtop CPC, part of the CPC Modeler platform — a compact, desktop-sized unit with mg to g/cycle loading capacity and high-purity output. It also includes the CPC Simulator tool, which helps identify optimal purification methods using partition coefficients, crude composition, and a digital method library.

The resulting fractions demonstrated impressive performance:

- ▶ Fractions 36–51 achieved 96.6% purity with a calculated yield of 94.5%
- ▶ Fractions 38–49 achieved 98% purity with a 74.5% calculated yield

Fractions were lyophilized to remove organic solvents, followed by alcohol precipitation to eliminate salts and residual additives. This study confirms that CPC enables high-purity, high-yield purification of oligonucleotides in a single step — without requiring a solid stationary phase.

To remove the organic solvents, fractions were lyophilized, and salts and residual additives were easily removed by alcohol precipitation method.

RESULTS

Results of the experiment are shown in Figure 3.

The purity and calculated yield was different, whether you examined a wider or narrower pool of the collected fractions. Examining fractions 36–51, the achieved purity was 96.6%, with 94.5% calculated yield.

Examining the narrower pool of fractions 38–49, the achieved purity was a higher 98% but had a lower 74.5% calculated yield.

The resulting purity was above 95% in all cases, allowing choice of priority between higher purity and higher yield.

CPC can produce a high purity, high yield product without the use of expensive solid stationary phase, in a single purification step.

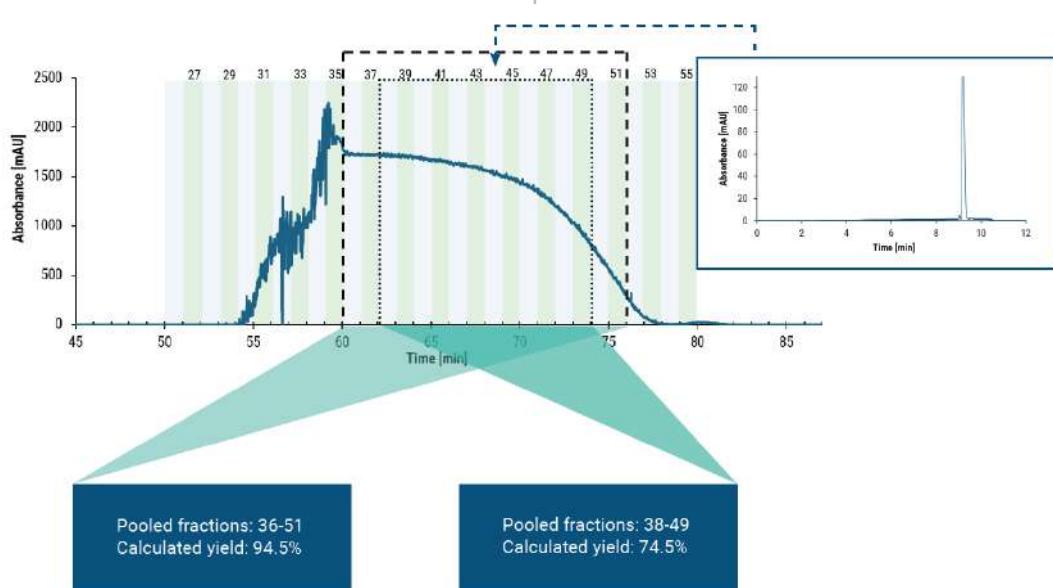


Figure 3: The chromatogram on the left shows the results of the CPC separation. The collected fractions (coloured stripes & numbers) and pooled fractions are marked. The smaller HPLC chromatogram on the right side shows the AEX-HPLC analytical chromatogram of fraction 44.

The CPC Modeler allows the validation of digital purification setups with the provided semi-preparative partition chromatographic equipment. The Benchtop CPC allows the testing of purification methods, and the fine-tuning of operational parameters for optimal performance.

Real-Time Monitoring of the Suzuki Reaction Using Compact Mass Spectrometry

Application of the Advion expression® CMS for Rapid, On-Bench Analysis and Workflow Optimization in Synthetic Chemistry

Reaction monitoring is a critical step in chemical synthesis, enabling chemists to determine the optimal time to quench reactions for maximum yield and purity. Advion's expression Compact Mass Spectrometer (CMS) offers a versatile, real-time solution for reaction analysis directly at the bench, integrating with a broad range of sampling techniques including LC/CMS, FIA, TLC via Plate Express®, Atmospheric Solids Analysis Probe (ASAP®), and inert ASAP® (iASAP®) for air-sensitive reactions. Demonstrated case studies—such as Suzuki couplings, 4-iodoisquinoline synthesis, and 6-iodotryptophan formation—highlight the CMS's ability to provide rapid, selective, and direct reaction monitoring without extensive sample preparation. This capability streamlines workflows, enhances decision-making, and maximizes efficiency in synthetic and medicinal chemistry research.

Keywords or phrases:

Reaction Monitoring, Compact Mass Spectrometer, Real-Time Analysis, LC/CMS Integration, ASAP Sampling, Synthetic Chemistry



expression®
High performance compact mass spectrometer

INTRODUCTION

Real-Time Reaction Monitoring Answers

Reaction monitoring is a key aspect in a range of chemistry environments from chemical synthesis to drug discovery to understanding natural products to protein synthesis. Understanding the optimal time to quench a reaction for maximum yield, as well as monitoring a reaction in real-time are vital to many medicinal and synthetic organic chemists. Advion's expression CMS was developed with the chemist in mind to optimize their workflow directly at the bench. It is an easy-to-use and maintain single quadrupole detector that integrates with the industry's broadest range of innovative sampling techniques from direct probe analysis to ultra-high performance liquid chromatography. Users can rapidly switch between the many different sampling techniques required throughout the chemist's workflow.

Real-time monitoring of a Suzuki reaction via LC/ CMS and FIA

Medicinal chemists are routinely faced with personally synthesizing over a hundred new chemical entities (NCEs) each year for testing as future pharmaceutical drug candidates. The goal is to prepare a high-yield, relatively pure product via an optimized synthetic route. TLC and LC/MS are routinely used to guide these reaction outcomes but are typically only available through a central core facility or a shared open access of systems. For real-time monitoring and rapid answers, the CMS can fit directly in a fume hood for hood-based applications in the analysis of chemical reactions employing either LC/CMS or flow injection analysis (FIA).



Figure 1: The CMS with reaction equipment directly inside of a fume hood.

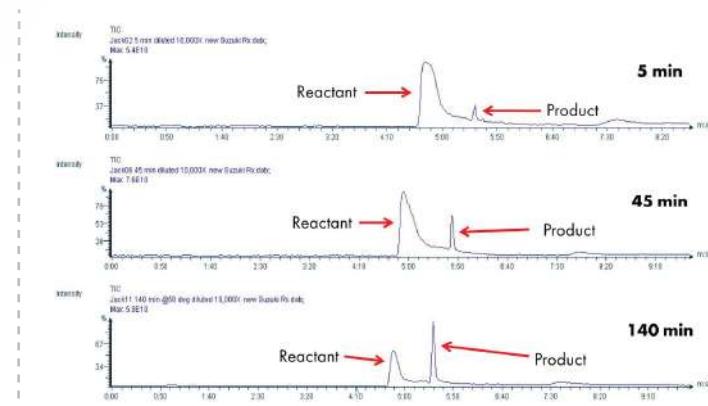


Figure 2: LC/CMS analysis of Suzuki reaction.

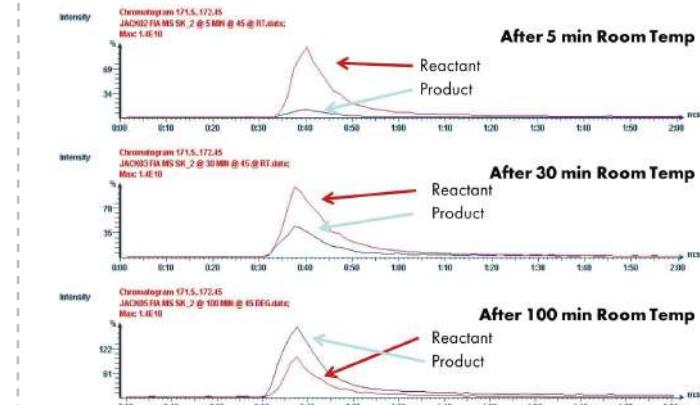


Figure 3: FIA analysis of Suzuki reaction.

The results shown for the LC/CMS analysis (Figure 2) and FIA analysis (Figure 3) of the Suzuki reactions to prepare p-Aminobiphenyl demonstrate proof of principle for real-time reaction monitoring. Both results show the increase of product/reactant ratio over time. Real-time monitoring of the reaction mixture with a selective detector optimizes the chemist's workflow to produce a high yield of the desired product in a minimal period of time.

Reaction Monitoring via TLC

Thin layer chromatography (TLC) is a simple, cost-effective technique that provides critical information about synthetic reactions, and is often employed for reaction monitoring in medicinal and organic synthetic labs. Advion's Plate Express® TLC Plate Reader seamlessly integrates with the CMS and provides compound structural information directly from TLC plates without additional preparation.

The results of a Suzuki reaction for the synthesis of 4-aminobiphenyl show an extracted ion chromatogram (XIC) of the increasing of the product ion and the decrease of the reactant ion over time demonstrating real-time monitoring. The reactant ion was no longer detected at 180 minutes indicating the reaction was complete. The CMS coupled with the Plate Express allows users to monitor reactions in real-time by evaluating the mass spectra for structural information directly from the TLC plate.



Figure 4: Experimental set up of the CMS and Plate Express.

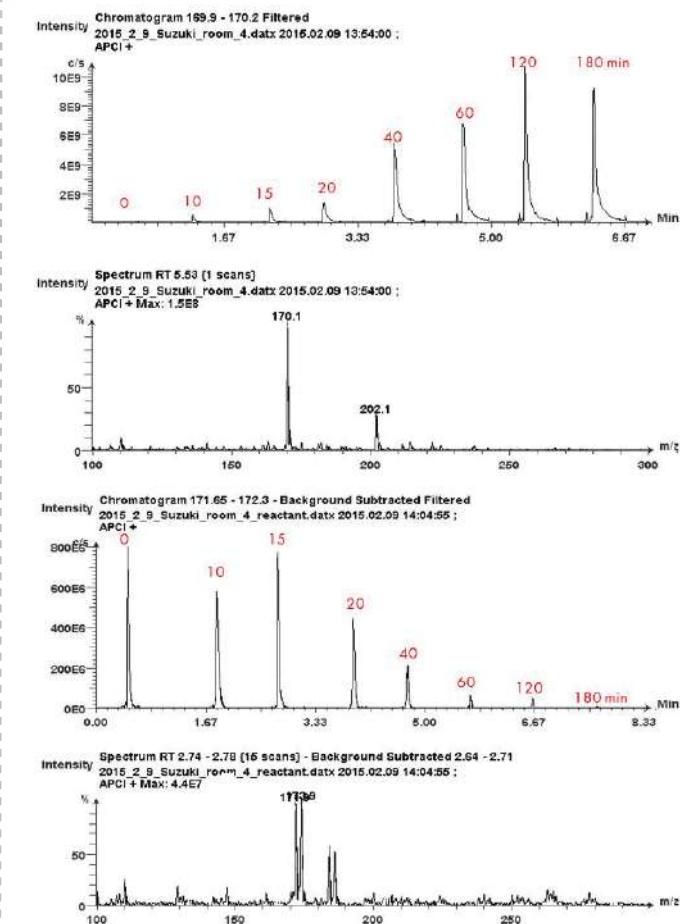


Figure 5: XIC of the synthesis of 4-aminobiphenyl.

Monitoring Synthetic Reactions via ASAP

Advion's Atmospheric Solids Analysis Probe (ASAP®) offers direct and instant mass analysis. Simply dip the ASAP into a liquid or swipe across a solid and insert the probe directly into the ASAP-enabled APCI ion source of the CMS for analysis (Figure 6). The ASAP requires no sample preparation, no chromatography, and provides sensitive analysis of a wide range of compounds in less than 30 sec.

Two experiments were demonstrated with the ASAP. In the first experiment, the synthesis of 4-iodoisouquinoline using the methods of Artis and Buchwald¹ could not be monitored by TLC. However, with the ASAP, the reaction product is readily seen after reacting at 110 °C for 22 hours at m/z 256.02 (Figure 7).

The results of the second experiment to determine the optimum stop time for the reaction based on the work of Yaetko et al^[2] show an increase in the formation of 6-iodotryptophan and the acetamide-protected version of 6-iodotryptophan product ions as the presence of 6-iodoindole reactant ions decrease (Table 1). The timed study show that the reaction was reaching a plateau at approximately 60 min, indicating the reaction could have been stopped at 60 min.



Figure 6: ASAP with sample inserted into the ASAP-enabled APCI ion source of the CMS.

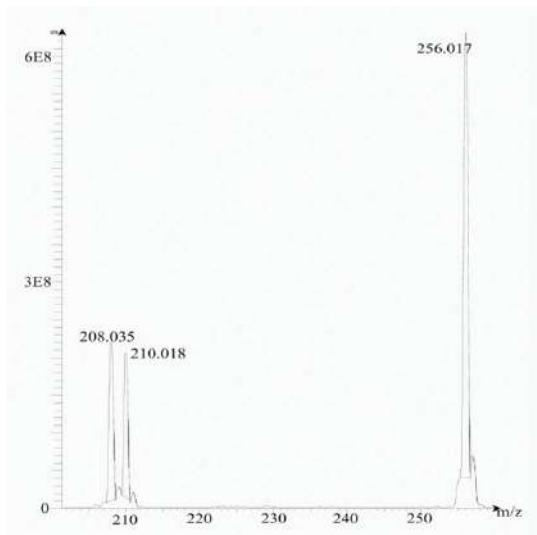


Figure 7: Mass spectra of the synthesis of 4-iodoisouquinoline.

TIME (MIN)	% 6-INDOLOINDOLE	% 6-INDOTRYPTOPHAN	% 6-INDOTRYPTOPHAN PROTECTED
1	99.73	0.15	0.12
30	94.44	4.62	0.94
60	20.20	65.17	14.63
120	15.2	60.67	24.12

Table 1: Presence of reactant and product ions over time.

Monitoring Air-Sensitive Reactions via iASAP

The inert ASAP (iASAP[®]) is a modification of this technique that allows for easy sampling of air-sensitive compounds from reactions directly from a glove box or Schlenk line (Figure 8). Metal based compounds have found utility in various fields such as clinical, energy, food safety and environmental to name a few. Creating the metal complex is the last step in a synthetic process, when ligands are bound to the metal centre. Once made, these metal complexes can be used anywhere from stereospecific synthesis to anti-cancer drugs. It is critical that reaction conditions are providing the desired product and that side products are kept to a minimum to maximize yield.

During synthesis for a Molybdenum complex, the product crystals precipitate from the solvent, making direct product monitoring difficult, but the formed reaction products remain in solution and can be monitored. When the reaction was exposed to air, the formation of lower mass Mo monomers were reduced (Figure 10) compared to when the reaction was protected from air (Figure 9). Even without directly monitoring product formation, the byproduct from the reaction indicates reaction progress.



Figure 8: The iASAP, an inert modification developed for the original ASAP, for CMS analysis of air-sensitive samples.

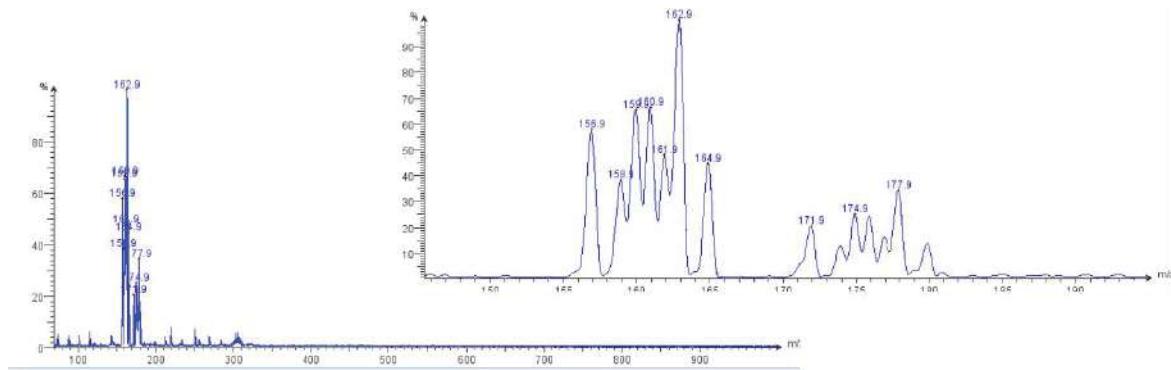


Figure 9: Mass spectra of reaction under nitrogen protection from air.

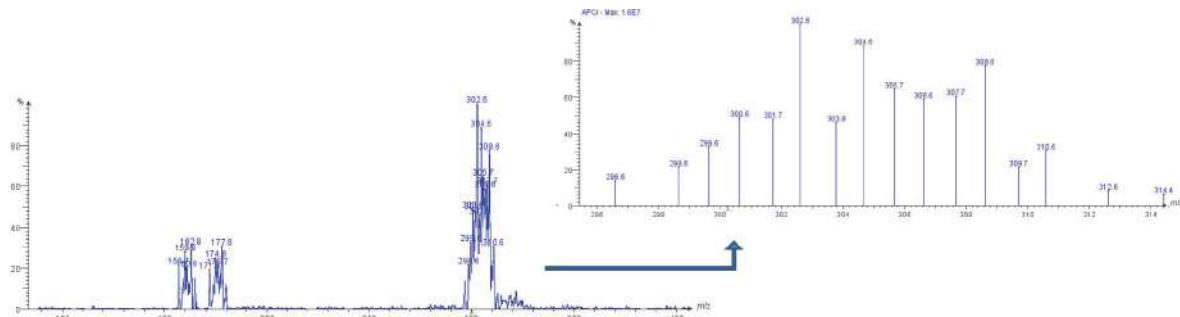


Figure 10: Mass spectra of reaction with air introduced.

CONCLUSION

Advion's expression Compact Mass Spectrometer (CMS) addresses the need for organic and synthetic chemists to understand the optimal time to quench a reaction mixture. The CMS fully integrates with the industry's broadest range of innovative sampling technique allowing chemists to obtain answers within minutes or even seconds.

REFERENCE

Compact Mass Spectrometry: A complete reaction monitoring solution, <https://www.advion-interchim.com/>



Analysis of Aggregates and Additives in Antibody Drugs

Shodex Solutions for Reliable Aggregate Separation and Surfactant Quantification

Ensuring the safety and efficacy of antibody therapeutics requires reliable methods for monitoring protein aggregation and additive content during production and storage. In this study, the Shodex PROTEIN LW-803 silica-based SEC column demonstrated superior separation of antibody monomers, dimers, and higher-order aggregates compared with competing columns, enabling precise characterization of IgG and BSA. The column maintained high resolution even at increased injection volumes, highlighting its robustness for quality control applications. Additionally, a rapid LC/MS method was developed using the polymer-based Shodex ODP2 HP-2D column for quantification of polysorbate surfactants (Tween 20 and Tween 80) in the presence of IgG and NaCl, without requiring pretreatment steps. The combination of high-resolution aggregate analysis with sensitive surfactant quantification provides a streamlined workflow for comprehensive QC of antibody drugs.

Keywords or phrases:

Antibody drugs, protein aggregates, Shodex PROTEIN LW-803, polysorbate (Tween) analysis, quality control (QC) in Biopharmaceuticals



PROTEIN LW series

Silica-based columns for aqueous SEC (GFC) analysis

INTRODUCTION

Antibody drugs are important potent therapeutic drugs. Since antibody drugs specifically target the cancer cells, they can cause less side effects. However, it is known that they may aggregate to form dimers and other larger aggregates during manufacturing process and/or during storage period. There are concerns that those larger aggregates may produce antibodies or become immunogenic conjugates that have properties of eliciting cell immune responses inside the body, which can lead to side effects. This may lead to side effects. Therefore, monitoring such aggregates is an important quality control (QC) criterion.

Silica-based aqueous size exclusion chromatography (SEC) columns are generally used for the separation of aggregates. Shodex PROTEIN LW-803 is one of them and it has high separation capability for the purpose. This application demonstrates the effectiveness of LW-803 by comparing it to similar columns from other companies. IgG (molecular weight (MW) about 150,000) and Bovine serum albumin (BSA, MW 66,000) were analyzed for the comparison. The effects of injection volume for the analysis of polyclonal IgG was also tested.

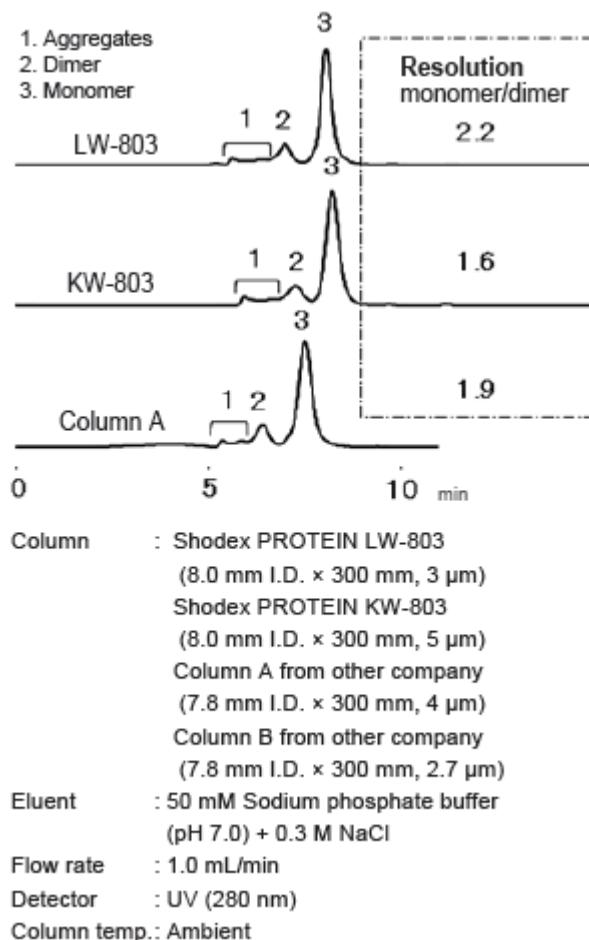
During the production of antibody drugs, small amounts of surfactants may be added to solubilize or stabilize IgG, and thus management of surfactants' concentrations is also important QC criteria. Quantification of surfactants can be difficult, as they exist as complex matrixes and have low UV absorption. In this application, we developed a rapid LC/MS method with a use of polymer-based column, Shodex ODP2 HP-2D, for the quantification of polysorbate (Tween) in the presence of IgG and NaCl as matrixes. The method does not require sample pretreatment prior to the injection.

1. COMPARISON OF SEC COLUMNS

1.1 Analysis of IgG

The test samples were prepared by dissolving polyclonal IgG from human serum or monoclonal IgG from recombinant

CHO cells in the eluent. Figure 1 shows the chromatograms of polyclonal IgG by injecting 5 μ L of 10-mg/mL sample. The monomer as well as dimer and even larger aggregates were detected. It can be seen that LW-803 demonstrated a superior separation efficiency between monomer and dimer compared to that of KW-803 and a column from another company, which is recommended for the analysis of antibodies.



Next, monoclonal IgG was analyzed by injecting 20 μ L of 1-mg/mL sample (Fig. 2). Similar to polyclonal IgG analysis, monomers as well as dimer, trimer, and even larger aggregates were detected. The resolutions between monomers and dimers and between dimer and trimer obtained by LW-803 were superior to that of columns from other companies.

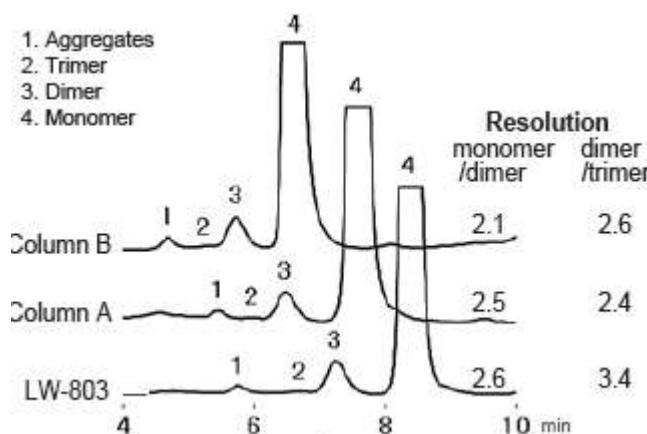
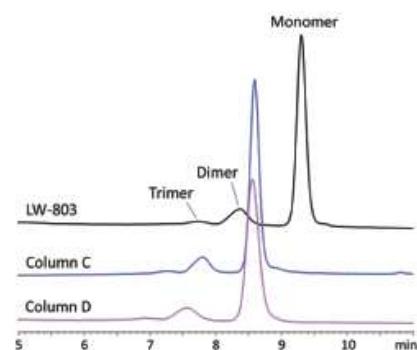


Figure 2. Chromatograms of polyclonal IgG

2. ANALYSIS OF BSA

Figure 3 shows the chromatograms of BSA obtained by injecting 5 μ L of 2-mg/mL sample. The monomer as well as dimer and trimer were detected.



Column : Shodex PROTEIN LW-803 (8.0 mm I.D. \times 300 mm, 3 μ m)
 Column C from other company (7.8 mm I.D. \times 300 mm, 3 μ m)
 Column D from other company (7.8 mm I.D. \times 300 mm, 5 μ m)
 Eluent : 50 mM Sodium phosphate buffer (pH 7.0) + 0.3 M NaCl
 Flow rate : 1.0 mL/min
 Detector : UV (280 nm)
 Column temp.: 25 °C

Figure 3. Chromatograms of BSA

Figure 4 is a superimposed chromatogram of the above three analysis by aligning the BSA monomer peaks.

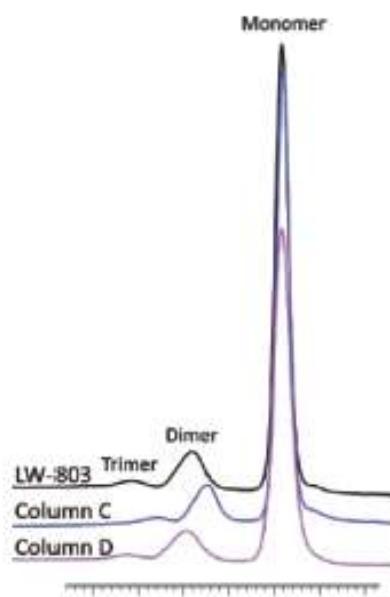


Figure 4. Superimposed chromatograms of BSA

Theoretical plate number (TPN) and resolution (Rs) between the monomer and dimer for each column are summarized in Table 1. It demonstrated that LW-803 achieved the highest resolution among the three columns studied.

	RT (min)	TPN	Rs (M/D)
LW-803	9.30	17,478	2.33
Column C	8.59	14,986	2.07
Column D	8.56	8,594	2.07

Table 1. TPN and Rs (monomer/dimer) for the analysis of BSA

2.1 Effects of sample injection volume

Effects of sample injection volume for the analysis of IgG was studied. The volume between 1 and 100 μ L of 10-mg/mL polyclonal IgG was injected to LW-803. Figure 5 shows chromatograms and a graph presenting the relationships between the injection volume and TPN and resolutions between monomer and dimer.

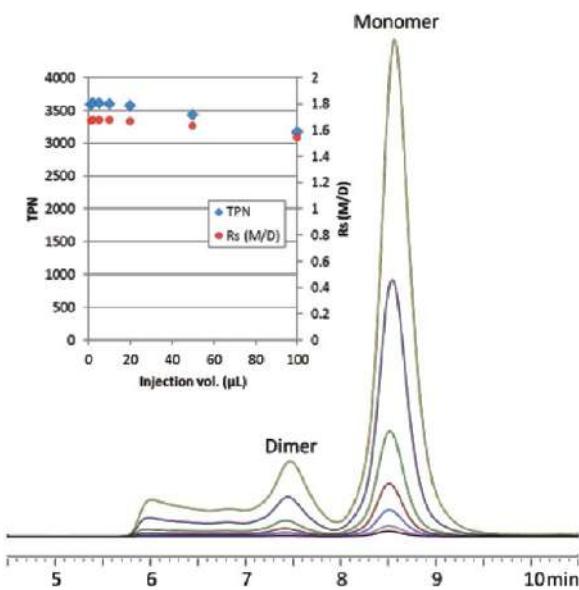


Figure 5. The effects of sample injection volume

The separation efficiency was consistent among all injection volumes in the 1 - 20 μL range. It also demonstrated the method's capability for handling larger injection volume (100 μL , absolute sample loading of 1 mg) as there was no significant decrease in its separation efficiency (Rs larger than 1.5). The Rs obtained in this experiment was lower than the data obtained in experiment 2.1. This is due to the differences in the analytic systems used. Thus, it is worth noting that the system's dead volume may affect the resolution.

3. ANALYSIS OF SURFACTANTS IN ANTIBODY DRUGS

ODP2 HP-2D is packed with polyhydroxy methacrylate gels. Unlike regular ODS columns, ODP2 HP is highly hydrophilic and this prevents proteins from being retained inside the column. Hydrophobic interaction which is the cause of protein retention can be suppressed almost to none by using alkaline eluent. Also, ODP2 HP's small pore size (40 \AA) adds a size exclusion effect that helps proteins to elute at v_0 . By controlling those features, the ODP2 HP column completely separates IgG from surfactants. The surfactants are retained by reverse phase mode. In order to prevent IgG from entering

the MS, a flow-switching valve was placed after the UV detector. By using the valve, the column eluate obtained between 0 and 5 minutes is collected in a waste bottle and the eluate obtained after 5 minutes is injected into the MS. Tween is typically used as an additive in antibody drugs; thus, we selected it as a test surfactant in this application. Tween consists of a sorbitan backbone, polymers of ethylene oxide (EO), and long chain fatty acids connected by ester bonding (Figure 6).

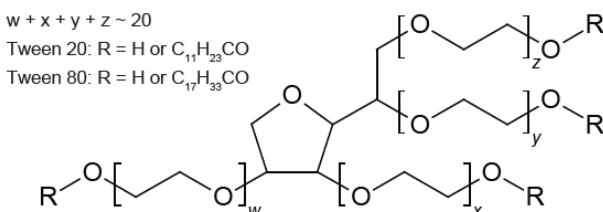
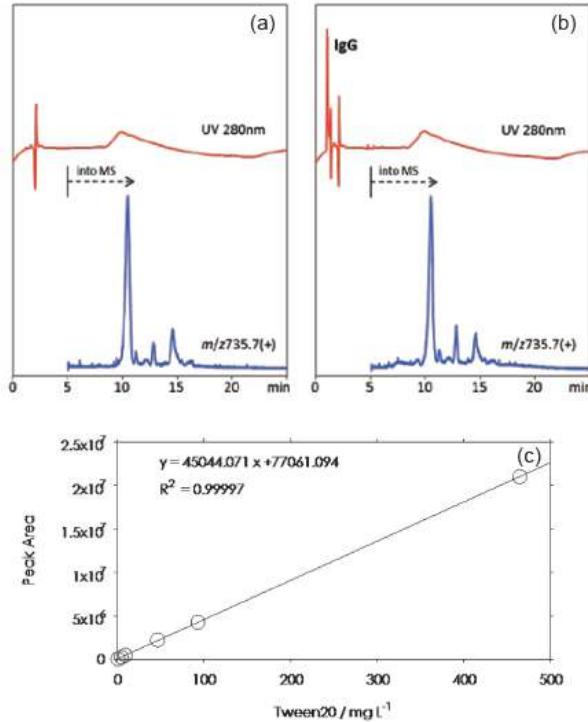


Figure 6. Structure of Tween

Tween does not exist as a single compound but exists in several different forms. The most abundant component was selected by pre-scanning the sample. Quantification curve was prepared using the result obtained by an MS (SIM mode) by measuring selected ions. Among the available Tween standards, Tween 20 and Tween 80 were analyzed. As a test sample, a mixture containing 50-mg/L Tween 20 or Tween 80, 1-g/L polyclonal IgG, and 0.3-mol/L NaCl was prepared. Injection volume used was 2 μL throughout the experiment.

3.1 Analysis of Tween 20

Figure 7 (blue line) shows the SIM chromatogram of Tween 20. The eluent used was ammonium water. Ion at m/z 735.7 was monitored. This ion is assumed to be a divalent ion consisting of a sorbitan backbone, 20 or more EO units, glycerol diesters (MW 1435) of C14 and C12 with either one of them having a double bond, and two ammonium ions. Two chromatograms, one including IgG and NaCl (Fig. 7(a)) and the other without IgG and NaCl (Fig. 7(b)) demonstrated very similar results, showing that there was minimum ion-suppression effect. Also, a good linearity was obtained for the calibration curve of Tween 20.



Column : Shodex ODP2 HP-2D
 (2.0 mm I.D. x 150 mm, 5 μ m)
 Eluent : (A) 0.1 % Ammonia aq./ (B) CH_3CN
 Linear gradient
 20 % B (0 - 5 min) → 20 to 90 % B (5 - 19 min)
 → 90 % B (19 - 24 min) → 90 to 20 % B (24 - 25 min)
 Flow rate : 0.2 mL/min
 Detector : UV (280 nm)
 ESI-MS SIM(+)
 Column temp.: 40 °C

Figure 7. Analysis of Tween 20. Chromatograms of (a) without and (b) with 1-g/L IgG and 0.3-mol/L NaCl. (c) calibration curve

3.2 Analysis of Tween 80

Tween 80 was analyzed using the same method as for Tween 20. Ion at m/z 848.8 was monitored (Figure 8). This ion is assumed to be a divalent ion consisting of a sorbitan backbone, 28 or more EO units, glycerol monoester (MW 1661) of C18 with a double bond, and two ammonium ions. Consistent with Tween 20's analysis result, the chromatograms of samples containing with and without IgG and NaCl were very similar, showing the presence of minimum ion suppression. Again, good linearity was obtained for the calibration curve of Tween 80.

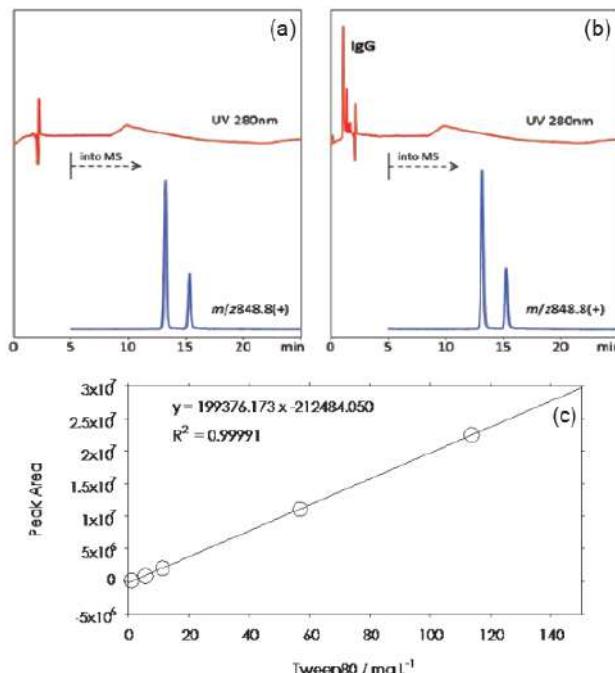


Figure 8. Analysis of Tween 80. Chromatograms of (a) without and (b) with 1-g/L IgG and 0.3-mol/L NaCl. (c) calibration curve.

CONCLUSION

Shodex PROTEIN LW-803 demonstrated its superior separation capability for IgG monomers and its aggregates. It also has high separation capability in even lower molecular weight ranges, and this allows the analysis of antibody metabolites and other proteins. Moreover, the column is capable of handling high loading volumes. Therefore, LW-803 has a wide usability for the separation of not only antibody aggregates, but for other proteins.

Moreover, Shodex ODP2 HP-2D proved its separation capability of analyzing non-ionic surfactants, Tween 20 and Tween 80, in the presence of IgG and NaCl. The method does not require the use of deproteinization nor desalting sample pretreatment. The method showed the effectiveness of the alkaline eluent for separating IgG at V_0 . The use of the alkaline condition was possible because of the polymer-based packing material used in ODP2 HP. The method developed in this application is fast and high-sensitive for the analysis of surfactants which would

be applicable in antibody drug QC analysis. Even faster analysis can be expected by using a shorter column of the ODP2 series, ODP2 HP-2B (2.0 I.D. x 50 mm L), and coupling it with MS.



Small Scale Peptide and Impurity Isolation Using the ACQUITY UPLC H-Class and Waters Fraction Manager-Analytical Systems

Precision Fraction Collection and High Reproducibility with Waters UPLC H-Class and WFM-A Systems

The ACQUITY UPLC H-Class System configured with the Waters Fraction Manager-Analytical (WFM-A), allows scientists to perform small scale peptide and impurity isolation with assurance. This application note illustrates the utility of the

ACQUITY UPLC H-Class and Waters Fraction Manager-Analytical (WFMA) Systems for the analysis and isolation of a synthetic peptide and its closely eluting impurities.

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Waters Corporation

Keywords or phrases:

Waters ACQUITY UPLC H-Class, Waters Fraction Manager-Analytical (WFM-A), peptide isolation, impurity analysis, small-scale purification

ACQUITY UPLC H-Class System with Waters Fraction Manager-Analytical (WFM-A) System.



INTRODUCTION

As peptides become more popular in the development of new therapeutics, it is increasingly important to quickly optimize the synthetic and cleavage processes by isolating and identifying both the target peptide and its related impurities. Collecting and analyzing closely eluting impurities while isolating the target peptide saves time and effort and provides additional information about steps that can be taken to improve the quality and yield of the peptide product. While peptide isolation is routine for groups involved in synthesis and cleavage, peptide isolation is also useful for scientists in research and discovery groups. Whether peptide studies are focused on how these complex molecules affect the body and are metabolized or on how peptides are isolated from naturally occurring sources¹, only small amounts are required for initial experiments.

This application illustrates the utility of the ACQUITY UPLC H-Class and Waters Fraction Manager-Analytical (WFM-A) Systems for the analysis and isolation of a synthetic peptide and its closely eluting impurities. This instrument configuration can be adapted for the isolation of constituents from complex synthetic, metabolic, or natural product mixtures at the small scale.

EXPERIMENTAL

- ▶ **Analytical column:** XBridge Peptide BEH C18, 4.6 x 50 mm, 5 μ m
- ▶ **Flow rate:** 1.46 mL/min
- ▶ **Mobile phase A:** 0.1% trifluoroacetic acid in water
- ▶ **Mobile phase B:** 0.1% trifluoroacetic acid in acetonitrile
- ▶ **Wash solvent:** 7:2:1 Acetonitrile/Methanol/Water
- ▶ **Purge solvent:** 9:1 Water/Methanol
- ▶ **Wavelength:** 280 nm
- ▶ **Gradients and injection volumes:** as noted in figures
- ▶ **Column temperature:** 30 °C

- ▶ **Sample:** Crude synthetic peptide comprised of the following 16 amino acid residues: 7 polar, 6 nonpolar, 1 acidic, 2 basic; purity 56% by HPLC

INSTRUMENTATION

- ▶ ACQUITY UPLC H-Class System with an ACQUITY UPLC
- ▶ PDA Detector and Empower 3 Software
- ▶ Waters Fraction Manager-Analytical

RESULTS AND DISCUSSION

The principles of scaling chromatography² remain the same whether the objective is to increase the amount of sample isolated at one time on a large column, or to decrease the amount of product based on the immediate need for material to perform experiments which answer pertinent questions quickly. For these studies, another new aliquot of the crude synthetic peptide sample used in previous work³ was isolated using the same optimized and focused gradient⁴, this time at a much smaller scale – on a 4.6 x 50 mm XBridge Peptide BEH C18 Column using the ACQUITY UPLC H-Class System configured with a WFM-A fraction collector. The crude peptide (2.4 mg) was dissolved in dimethylsulfoxide (DMSO) and filtered using a 13 mm Acrodisc GHP syringe filter. Whereas 10 μ L was the maximum injection volume that maintained resolution between the peptide and its impurities on the 4.6 x 100 mm XBridge C18 Column used in previous work (Figure 2), geometric scaling to the shorter 4.6 x 50 mm column for these experiments reduced the injection volume to 5 μ L. Likewise, the reduction in column length automatically reduced the gradient run time from 18 minutes on the 100 mm column to 9 minutes on the 50 mm column. With the target peptide peak and its closely eluting impurities eluting well before 36% B, the gradient conditions were adjusted to run from 28–32% B in 5 minutes, thus saving time in the method. Because of the complexity of crude synthetic peptide samples, shallow focused gradients with slopes of about 0.2–0.3% change per column volume are useful for resolving more sample constituents.

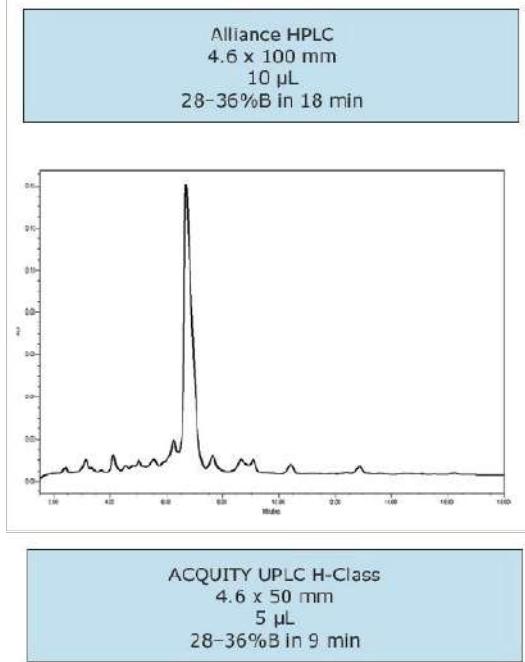


Figure 1. The peptide sample profile was very similar on two different systems.

Geometric scaling principles were applied. The sample concentration for work done on the Alliance HPLC System was 7.2 mg/mL while the sample concentration used on the ACQUITY UPLC H-Class System was 2.4 mg/mL. Column chemistry: XBridge C18, 5 μ m.

The ACQUITY UPLC H-Class System, with its low system dispersion⁵, exact control of solvent composition⁶, and accurate sample injection scheme⁷, provided excellent chromatographic reproducibility, as shown in Figure 2, where five peptide injections overlaid exactly. The WFM-A was specifically designed to minimize peak dispersion during collection. Figure 4 emphasizes the benefit of low

peak dispersion in the fraction collection valve with the comparison of chromatographic profiles obtained with the WFM-A and with a traditional collector.

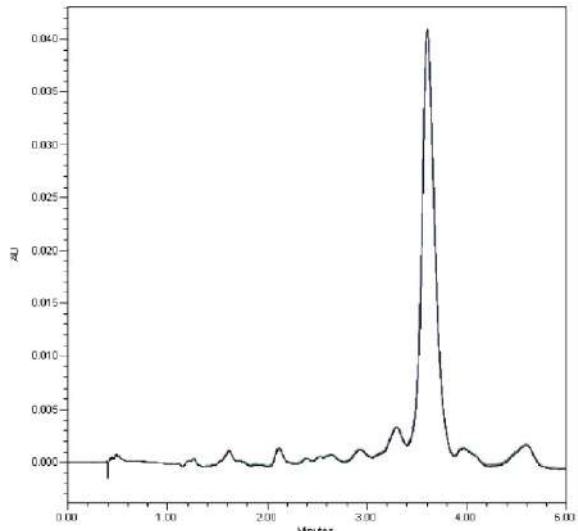


Figure 2. Overlay of 5 peptide injections on the 4.6 x 50 mm XBridge Peptide BEH C18 Column. Gradient: 28–32% B in 5 minutes, 5 μ L injection, 280 nm.

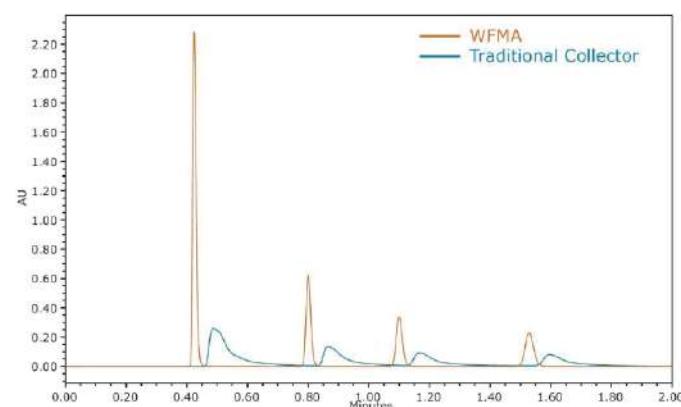


Figure 3. The WFM-A is specifically designed to address the challenge of peak dispersion. As shown, the WFM-A allows for the collection of concentrated narrow peaks of interest with the highest recovery possible.

Narrow, concentrated peaks are easily identified and collected with higher recovery when peaks are clearly defined. Fractions can be collected by time, slope, threshold, or any combination of the three. While the collection starting and ending times may be manually entered in the WFM-A method editor if desired, it is also possible to populate the WFM-A method automatically using the processed results

from an analytical injection (Figure 4). Selecting the result displays the integrated peaks with their retention times, start times, and end times, which are then automatically filled into the collection event table (Figure 5).

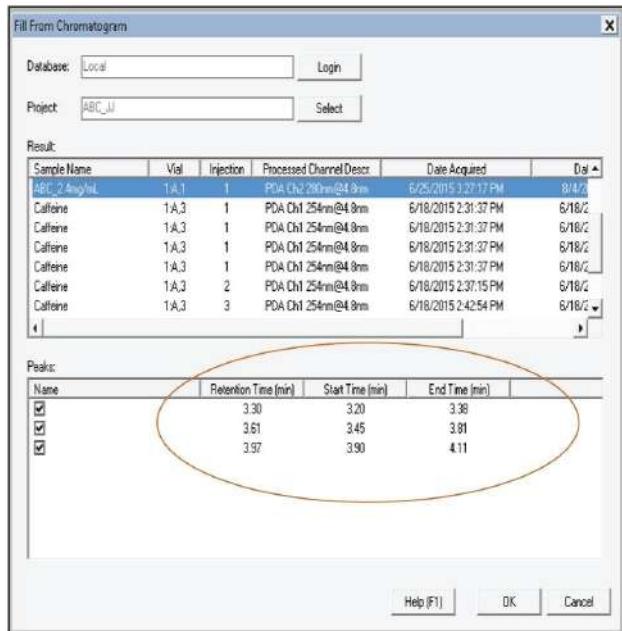


Figure 4. The sample result selected populates the peak table with the retention time as well as the start time and end time for each peak. Selecting OK fills in the Collection Event Table.

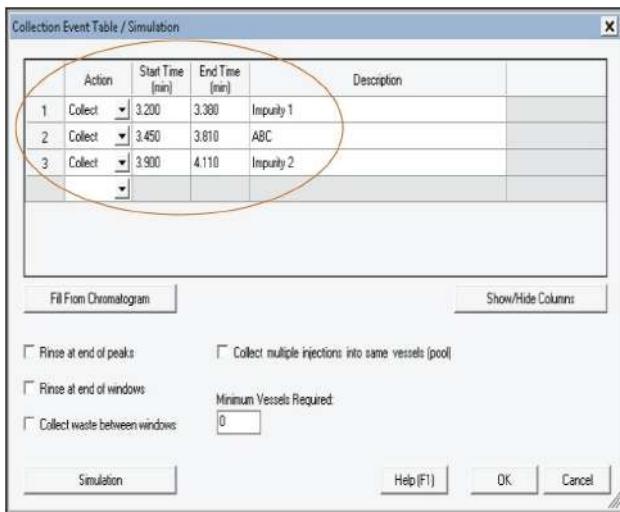


Figure 5. Collection Event Table in the fraction method.

With the collection method developed, the peptide and two closely eluting impurities were isolated from the crude sample in a total of ten injections. Because the ACQUITY UPLC H-Class System is so reproducible, all of the sample purification chromatograms were identical. Figure 6 shows a representative chromatogram with the shaded areas indicating where fraction collection occurred. The fraction volumes were essentially identical for each of the compounds in each of the isolations (impurity 1, 0.29 mL; peptide, 0.55 mL; impurity 2, 0.33 mL). All of the fractions of each type were pooled. An aliquot of each pool was immediately analyzed using two different gradients.

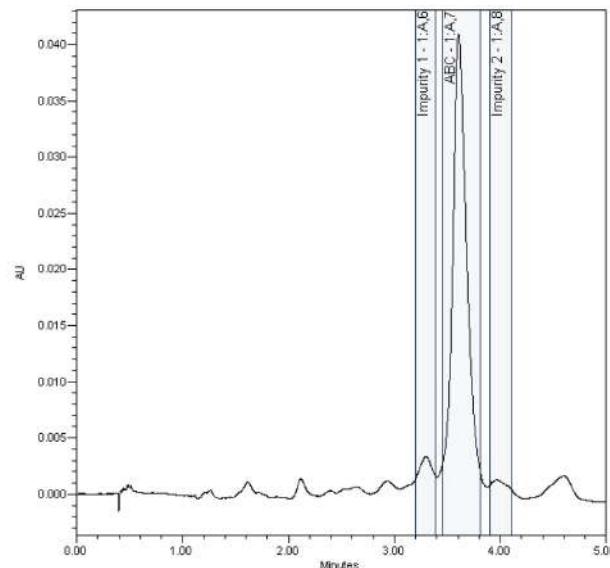


Figure 6. The peptide product and two closely eluting impurities were collected into a 48-well plate containing 2 mL vials. Gradient: 28–32% B in 5 minutes on a 4.6 x 50 mm XBridge Peptide BEH C18 Column, 5 μ m; 5 μ L injection; 280 nm.

The peptide product purity was 100% as determined by both the fast gradient (Figure 7) and the shallower focused gradients (Figure 8) used for fraction analysis. Slight differences in the purities of both of the contaminant peaks were evident as shown by the two gradients. While the fast gradient (3.38% change per column volume) showed impurity 1 to be about 83% pure, the shallow focused gradient (0.30% change per column volume) resolved yet another coeluting peak and reduced the estimated purity to about 77%.

Impurity 2 had a purity of 98% using the fast gradient and 80% using the focused gradient, again due to better resolution of compound constituents. If higher purity contaminant fractions were required for subsequent studies, further method development would likely be needed.

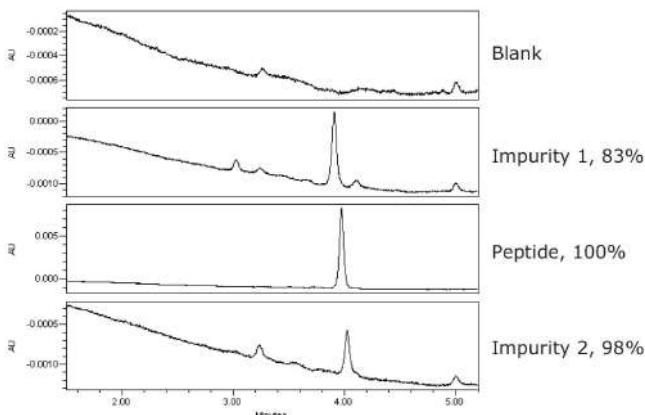


Figure 7. Approximate compound purities after subtracting the peaks present in the blank. Fraction analysis gradient: 5–50% B in 5 minutes, rate of gradient change 3.38%/column volume, injection volume 40 μ L, 280 nm.

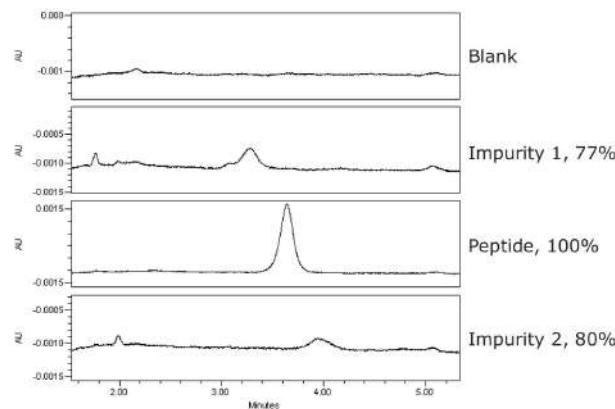


Figure 8. Approximate compound purities after subtracting the peaks present in the blank. Fraction analysis gradient: 28–32% B in 5 minutes, rate of gradient change 0.30%/column volume, injection volume 40 μ L, 280 nm.

CONCLUSION

The ACQUITY UPLC H-Class System configured with the Waters Fraction Manager-Analytical (WFM-A), with its very low system dispersion, exact control of solvent composition, accurate sample injection, and precise fraction collection,

allows scientists to perform small scale peptide and impurity isolation with assurance.

- ▶ Fast valve switching and movement between vessels, as well as a fraction divert valve with very low dispersion volume, facilitates narrow target peak collection and increases confidence in compound isolation.
- ▶ Small scale peptide isolation saves sample, time, and resources, promoting efficiency in the purification process.
- ▶ The ACQUITY UPLC H-Class System configured with the WFM-A can be adopted for the isolation of compounds from complex synthetic, metabolic, or natural product mixtures at the small scale.

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Updates

Recent Novel FDA Approved Drugs

Rilzabrutinib

What it is

Rilzabrutinib is a novel, oral Bruton's tyrosine kinase (BTK) inhibitor developed by Sanofi. Unlike earlier BTK inhibitors used in cancer, rilzabrutinib is reversible and covalent, designed specifically for autoimmune and inflammatory conditions.

How it works

BTK is a key enzyme in signaling pathways that drive B-cell activity and immune responses. By selectively and reversibly inhibiting BTK, rilzabrutinib dampens abnormal immune activation while preserving essential immune defense — a fine balance crucial in chronic autoimmune disorders.

Why it matters

Autoimmune diseases like immune thrombocytopenia (ITP) and pemphigus vulgaris (PV) often rely on broad immunosuppressants such as steroids, which carry significant side effects. Rilzabrutinib promises a targeted, oral alternative that can improve outcomes, reduce relapses, and offer patients better quality of life.

Donidalorsen

What it is

Donidalorsen is an investigational antisense oligonucleotide (ASO) therapy developed by Ionis Pharmaceuticals. It is being studied for hereditary angioedema (HAE), a rare disorder marked by sudden, painful, and sometimes life-threatening swelling attacks.

How it works

Donidalorsen targets the prekallikrein (PKK) gene, reducing production of plasma kallikrein—a key protein that drives the swelling cascade in HAE. By silencing this pathway, it helps prevent attacks before they start.

Why it matters

Current HAE treatments often involve frequent injections or infusions. Donidalorsen, delivered as a once-monthly subcutaneous injection, has shown the ability to dramatically cut attack rates with a favorable safety profile. For patients, this means more convenience, fewer disruptions, and better quality of life.





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