Volume 19
March 2015

3–4

56132

G.I.T. LABORATORY JOURNAL EUROPE

SCREENING TRENDS IN DRUG DISCOVERY inside

huber

GIT VERLAG
A Wiley Brand

SINTERGLASS

Borosilicate glass 3.3 with high chemical, thermal and mechanical resistance: Custom sintered elements made from VitraPOR® are characterized by their neutral properties and open-pore structure. Even for the most unusual applications.

VitraPOR®

Our VitraPOR® glass filters are heat resistant up to 540°C and can withstand most acids, alkalis and solvents.

They can be used in many chemical, pharmaceutical, biological and technical processes, and they are produced in porosity classes from under 1 µm to over 500 µm. VitraPOR® glass filters are always produced according to international standards, as are the custom-made solutions available in many shapes and sizes.

ROBU®

ROBU-GLAS® FILTER
Made in Germany

www.robuglas.com

Find Your Targets,

This year's Pittcon was somehow strange. On the one hand there was an extraordinary scientific program that attracted roughly the same number of scientists and thereby possible customers for the exhibitors like in the past. By the way, the number of scientist attending Pittcon is almost constant since the best attended Pittcons in the 90s. Only the number of booth personnel declined after the Lehman crisis. The overall number of participants in 2015 was roughly the same as in the past year, despite the fact that New Orleans lies in the south of the USA and the numbers of visitors in the south are always smaller than in the north eastern states.

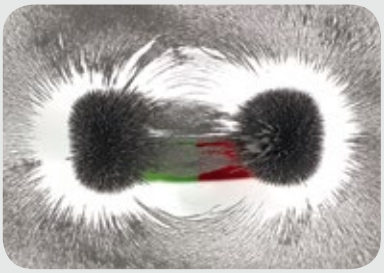
On the other hand visitors saw an exhibition consisting only of small and very small booths. Most "big" companies in the laboratory business cut down their booth sizes or were even completely absent. The booth space sold stays almost constant and the gaps left by the smaller booths were filled by smaller companies from the US, Europe, China, and India, who still see the US as an interesting market. They feel that spending money to get or stay in touch is a worthwhile exercise, especially at the biggest and most important conference and trade show on analytical chemistry in America.

On the question why they cut their expenses for presenting their innovations at Pittcon, the key personnel of a number of companies stated that this event is the most important event in the world to see what's new, to look for innovative companies for partnering or acquisition, but they feel that it is better to launch new products in an environment exactly fitting the precise target group of a given product, namely smaller events. Additionally their idea is not to wait for the next Pittcon to launch new products, but to look for the fastest way to market. The same people say that they appreciate the high quality of discussions and the presence of key personnel from other companies but stated that they plan to send less (key) personal next year.

Most of the big companies increasingly address well defined industrial markets like food-, petrochemical-, healthcare-, and environmental testing industries. Based on their existing technical platforms they "update" their instruments to "push one button- single purpose"-machines instead of investing in the development of new and better technology. Thereby they are in danger to ignore the needs of the academic market, a market which will be strongly fueled - at least in Europe by the 315 billion € investment program for IT infrastructure and research and education by the European Union. In general European markets are judged as not so important. In my opinion this is an underestimation. I see substantial growth in Germany and in most other European countries, especially in the laboratory markets, fueled by export friendly currency rates and a strong increase environmental- and food safety testing. If US companies want to get a piece of this cake, they should invest to reach the European markets now, if they do not – others will; and you can read above who this will be

Dr. Arne Kusserow
Editor-in-Chief

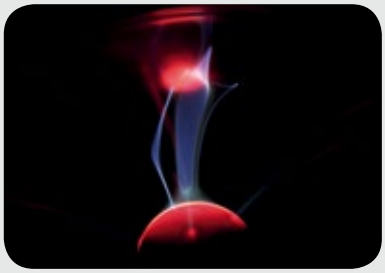
Content



Below 1K Page 12

Capacitive Deionization Page 17

X-Ray Free Electron Lasers Page 20



Creating Biointerfaces Page 23

Water Contaminants Analysis Page 30

Applied NMR Spectroscopy for Chemists and Life Scientists

Although it might be the most important analytical method for structure determination in chemistry, NMR spectroscopy was often introduced in the university curriculum from a theoretical point of view. Due to the ongoing rapid development of the technique, enabling applications beyond small molecules (all classes of biomolecules and monitoring of molecular interactions as prominent examples), also the requirements for a timely introduction of NMR spectroscopy changed fundamentally. Nowadays, direct transfer of theoretical models to experimental practice is mandatory. Oliver Zerbe and Simon Jurt follow in their textbook consequently this unique approach: The necessary quantum mechanical background is introduced at an appropriate level to understand all relevant new concepts such as multidimensional methods.

Simon Jurt

Prof. Dr. Oliver Zerbe

Why did you choose an application-oriented approach to writing this book?

The purpose was to provide all scientists who actually record spectra themselves, who want to understand basic NMR theory up to a fairly advanced level, or who need help in the interpretation of spectra, with the necessary knowledge.

How viable are benchtop NMR spectrometers for standard analytics?

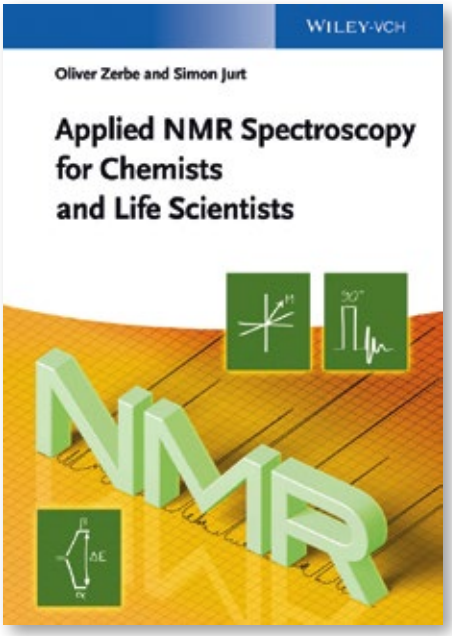
We have no experience with benchtop systems here in Zurich. However, we feel for our purpose here in chemistry – both in teaching as well as in research - we need full spectrometers capable for performing all standard experiments (1D and 2D experiments). We actually use 300 and 400 MHz spectrometer for student training in the advanced practical chemistry courses.

What was the reason to write this book?

We felt there is a need for a book that covers both practical as well as theoretical aspects of NMR and takes care of the fact that students measure spectra themselves (in contrast to the situation 20 years ago where samples were handed into a service). We also wanted to report on assignment techniques and strategies, an often-neglected aspect. The book should be suitable for beginners but be sufficient for all chemists and other scientists interested in this technique (except for those who do a PhD in the field of NMR)

Which background information does the reader need?

The reader needs no special background; all topics are developed from scratch. However, we admit that some basic understanding of principles in physical chemistry would be helpful. Some readers with very limited background may not understand every chapter, but we strongly feel that everyone interested in NMR will benefit.



Prof. Dr. Oliver Zerbe
is the head of the NMR department and group leader at the University of Zurich since 2003. He studied chemistry and obtained his PhD in the group of Wolfgang von Philipsborn in Zurich. Upon a postdoctoral stay in the group of Kurt Wüthrich, he conducted his habilitation in Medicinal Chemistry with Gerd Folkers. His main interests are structures of proteins, particularly of membrane proteins.

Simon Jurt
has been working for more than ten years in the NMR department of the University of Zurich after studies in chemistry at the University of Applied Sciences in Bern. He is responsible for maintenance of NMR spectrometers and practical NMR courses.

1. Edition December 2013
548 Pages, Softcover
ISBN 978-3-527-32774-4
Wiley-VCH, Weinheim
www.wiley-vch.de

Sample Chapter: http://bit.ly/NMR-Spectroscopy

SHIMADZU
Excellence in Science

Smart
Productivity
Operation
Performance

Demonstration lab at
Shimadzu Europa GmbH,
Germany

So Smart it almost runs itself!

GCMS-TQ8040 – Smart enough for everyday use in your laboratory

Smart Productivity

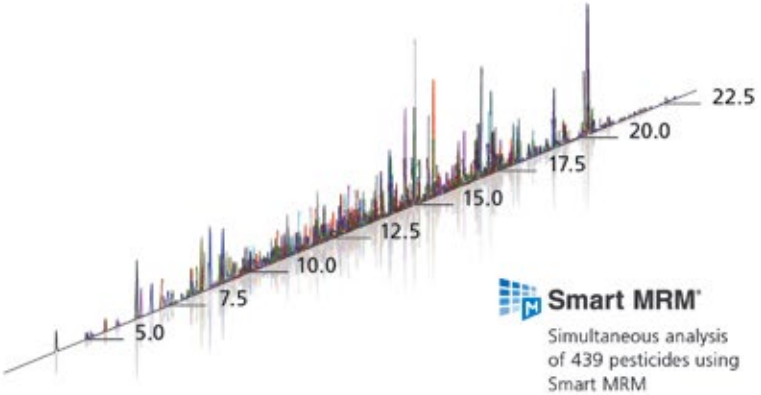
- 400+ compounds in one run
- Automatic method creation

Smart Operation

- MRM Optimization tool
- Smart Database series

Smart Performance

- Scan/MRM acquisition mode
- ... and much more!



Smart MRM®
Simultaneous analysis
of 439 pesticides using
Smart MRM

www.shimadzu.eu

NMR in Chemistry and the Life Sciences

New Challenges and Opportunities

Nuclear magnetic resonance spectroscopy (NMR) has always constituted a central analytical technique in chemistry. Previously, experiments were performed and interpreted by NMR experts. Keeping the instruments running was a central task of dedicated technicians. Teaching how to interpret NMR spectra based on chemical shifts and scalar couplings was a central task in the curriculum. The investigated structures usually fell into the small-molecules category. This has all dramatically changed in the meantime.

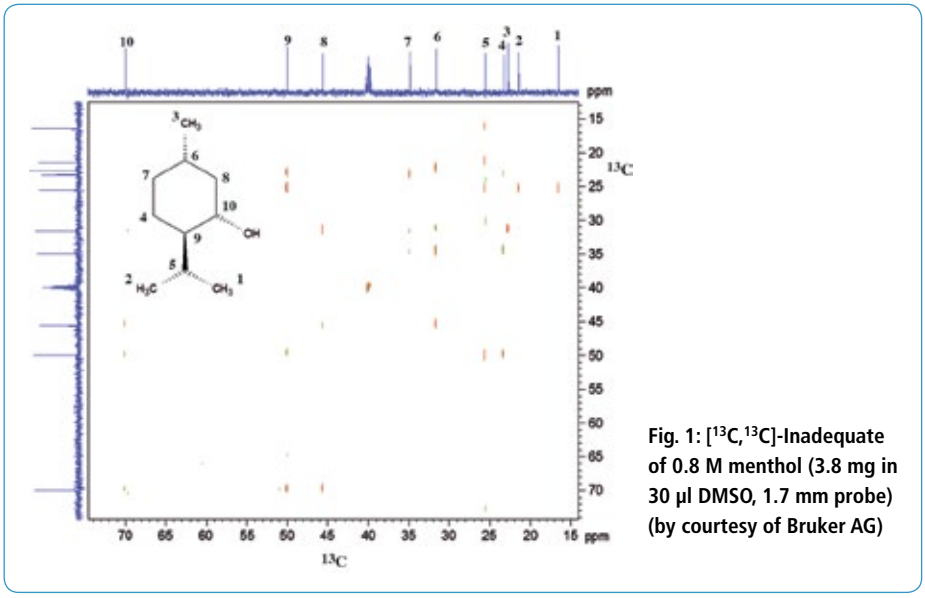


Fig. 1: [^{13}C,^{13}C]-Inadequate of 0.8 M menthol (3.8 mg in 30 µl DMSO, 1.7 mm probe) (by courtesy of Bruker AG)

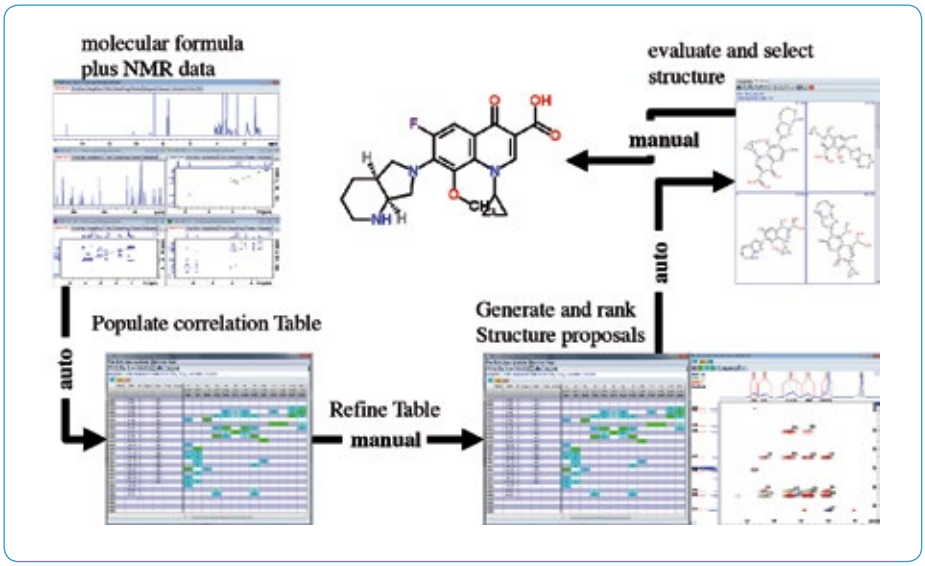


Fig. 2: Workflow during automatic structure elucidation from NMR data (by courtesy of Bruker AG)

Students in the Department of Chemistry at the University of Zurich (UZH) record NMR spectra on their own. They elucidate structures from first principles using correlations in 2D spectra. Instruments are used in equal share by researchers from inorganic, organic or physical chemistry. Most of the investigated molecules still belong to the class of small molecules but a large portion of instrument time is used for biological macromolecules. Studies comprise projects to determine protein or RNA structures, interactions of drug-like small molecules with proteins or interactions of metals with RNA. Chemical reactions are observed in the NMR tube, and variable-temperature NMR is exploited to extract activation energies. In short: The whole field has changed completely.

NMR has also sneaked quietly into other branches of science, in particular biochemistry and biology or materials science. Biology students here at UZH have been through chemistry and physics courses and are well-prepared to enter the field of NMR if desired.

A basic NMR setup requires a number of steps like locking, shimming and probe tuning/matching as well as calibration of pulses. These steps used to be fairly complicated, but run more or less in automatic fashion nowadays. However, students should have a basic understanding of these procedures. Because 2D NMR experiments play an essential role they also need to understand how these experiments work.

Practical Aspects of Analyzing NMR Spectra

The literature is full of excellent NMR books but often go far beyond of what is required for a typical user in chemistry or biology, while regularly failing to cover the practical aspects of performing NMR experiments. Another shortage is often that they do not really suggest strategies for assigning spectra and for establishing covalent structures. In principle the experiments used for analyzing spectra of peptides, steroids and oligosaccharides are similar, but the approach to assign them is often rather different, and the success depends on using the best set of spectra, and starting the assignment from the proper place. Based on lecture courses at the UZH a book has been published recently [1], in which a thorough description of the basic NMR phenomenon, a detailed account of frequently used NMR experiments, a description of the practical aspects of NMR, and a section on assignments strategies for natural products and biopolymers are provided.

At UZH students once they have received a basic training are allowed to use the web-based NMR booking schedule, and are free to measure their own spectra. The philosophy is to provide a good training and then encourage them to measure their own spectra. In the following experiments are described which are applied to typical problems from chemistry and biochemistry in the NMR department at UZH.

Technical Advances

The principle of NMR spectrometers is still the same but a number of technical advances have made their operation much easier. For example, shimming, the adjustment of field homogeneity, frequently required time-consuming optimization. Nowadays, gradient-shimming, a method that directly images field inhomogeneity within the sample, works in automatic fashion. The introduction of pulsed magnetic field gradients [2], has had a large impact on the quality of certain spectra, and now helps obtaining nearly artifact-free spectra. Another consequence of using gradients is that the number of scans per increment in 2D experiments is mostly limited by the signal/noise. Single-scan proton-carbon correlations often give nice spectra and can be recorded in less than 10 minutes. Recently, methods for fast acquisition of multidimensional spectra have been introduced. One set of experiments use so-called sparse sampling. In the sparse sample (also non-uniform sampling (NUS) experiments) not all of the data points are measured but a fraction of them, and the missing ones are reconstructed using mathematical methods [3]. Another set of experiments has been developed in which experiments are repeated very rapidly [4,5]. Normally, a relaxation delay after each scan secures that the perturbed spins have returned to equilibrium, but the length of this delay can be significantly reduced when selective pulses are applied. Thereby the overall experiment duration is cut down to one-tenth, often allowing complete 2D spectra to be recorded within seconds for isotope-labeled samples.

Sensitivity is the primary bottleneck of NMR, and some advances have been made to increase signal to noise ratio (S/N). One (unfortunately expensive) development are the so-called cryo-probes, that bring down the thermal noise resulting in a S/N that is often 4 times larger [6]. An experiment that could have lasted a week can now be done in just one night! Other methods to increase S/N is the usage of other NMR tubes, either with smaller diameter (3 mm, 1.7 mm or 1 mm) or susceptibility-matched glass inserts so that in mass-limited cases the concentration will be much higher within the NMR-active volume of the probe. The combination of all these advancements allows to record experiments when this was completely impossible before. A good example is presented by the INADEQUATE experiment in Figure 1, a carbon-carbon (natural abundance ^{13}C) correlation experiment, that used to require at least 50 mg of a small molecule compound, and which now becomes accessible with 10 mg or even less (in this case with approx. 4 mg).

Typical Small-Molecule Applications

The vast majority of spectra recorded at UZH still present 1D proton or carbon spectra, often using automatic sample changers. Other nuclei such as ^{19}F, ^{31}P, ^{29}Si, ^{11}B or ^{195}Pt (as part of inorganic complexes for catalysis or medicinal applications) are also measured quite frequently.

Chemists mostly know what the molecular formula of the expected reaction product looks like. However, when this is unclear, *de-novo* structure elucidation is necessary. This usually requires 1D proton and carbon spectra (also to check the purity) plus a standard set of 2D NMR spectra, mostly COSY, TOCSY, ROESY (or NOESY), [^{13}C,^{1}H]-HSQC and [^{13}C,^{1}H]-HMBC data [1]. The latter two experiments are the proton-carbon correlations along the one-bond coupling and the long-range couplings, respectively. The incorporation of ^{13}C shifts into assignments is mandatory for highly substituted compounds, and facilitates structure determination in general, in particular when using automatic methods (*vide infra*).

Software has been developed to evaluate spectra in a more automatic fashion. Peak multiplets are automatically evaluated to yield scalar couplings and chemical shifts and used to uncover correlations in spectra. In particular when incorporating 2D data, programs can nowadays propose complete structures of small molecules with good reliability. Figure 2 displays the workflow used for semi-automatic structure identification in commercial Bruker software.

The mantra of NMR analysis was the requirement for pure compounds. Sometimes reactions are monitored in the NMR tube, and the spectra will inherently contain compound mixtures. A method to separate a compound mixture into its constituents is diffusion-weighted measurement [7]. In the experiment signal losses are proportional to the fraction of molecules that have diffused significantly far away from their original position and hence are stronger for small than for large molecules. Such experiments can be recorded in a pseudo-2D fashion (called DOSY). In favorable cases sub-spectra of the individual molecules can be obtained.

Solid-state NMR spectroscopy used to be a playground for physicists. Recent technical advances have also helped to make the experiments simpler to perform. Disciplines of chemistry interested in solid-sate NMR spectra are those from material sciences or researchers in (heterogeneous) catalysis.

NMR is one of the methods suitable for determining activation energies of exchange processes [1,8]. At UZH a lot of variable temperature NMR spectra are recorded. Observing the change in peak position and linewidths as a function of temperature (or the determination of the coalescence temperature) allows extracting the reaction rates and the activation energy for the exchange process.

Applications in Life Science and Biochemistry

The usefulness of NMR is increasingly being recognized in biology and biochemistry as well. The determination of a protein structure is a considerable task, and often such a project, depending on the size of the protein and how well-behaved it is, can take a few years. The time-consuming step is the assignment of chemical shifts using data from 3D triple-resonance experiments.

Structures of proteins exceeding 40 kDa are usually tough projects and require producing a lot of specially labeled protein samples. One can resort to ^{15}N labeling even for small proteins (say even less then 50 residues, if there is a route for recombinant production), ^{15}N, ^{13}C double labeling in the range of 50-180 residues, while deuteration is required for those that are even larger. Figure 3 shows an example from research at UZH: It was discovered that the designed Armadillo repeat protein of the YM_3A type, in which Y and A denote the N- and C-terminal caps and M the three internal (identical) repeats, can be reconstituted from two complementary fragments. The spectrum of YM_2 on the top left clearly resembles a molten-globule type protein, and adding the unlabeled MA fragment converts that spectrum into one of a properly folded protein. The structure of the complex was determined based on NOEs.

Advances in spectroscopy have pushed the size-limit considerably. A major breakthrough was the development of the so-called TROSY-type pulse sequences [9], that allow to largely reduce the impact of the direct dipolar coupling for relaxation. To be most efficient all other protons should be removed by deuteration [10]. Lewis Kay has demonstrated that TROSY-type experiments can be used to assign the mega-Dalton assembly of the proteasome [11]. While this is certainly something for a highly specialized lab, the take home message here is that recording ^{15}N-^{1}H (or ^{13}C-^{1}H for methyl groups) correlation experiments are possible even for large proteins. Assigning them will be difficult, but sometimes this is not necessary, e.g. when screening for ligand-protein interactions. In fact, there are a number of biochemical problems that do not require extensive NMR expertise but just access to a spectrometer and a couple of hours of measuring time!

Even for the non-professional there is a lot to be discovered from simple protein NMR spectra: The chemical shift dispersion in 1D proton spectra will report on whether a protein is folded or not, and whether it is well-behaving (non-aggregating) at NMR concentrations. Signals from flexible residues are much more intense for large proteins. A typical question could be whether the expressed protein construct contains long flexible tails that hamper crystallization. If the protein can be produced in ^{15}N-labeled form a proton-nitrogen correlation map will even allow to quickly identify flexible parts and their location. When the protein is produced in *E. coli*, producing the ^{15}N-labeled form is usually easy, comparably cheap and worth the effort, because so much more information can be extracted from the 2D spectra.

Dynamic Studies

A fair amount of protein dynamic studies are also performed at UZH [12,14]. The backbone dynamics data are derived from ^{15}N relaxation experiments. The data analysis is rather straightforward, and it adds valuable data to the function-dynamics topic. One of the experiments recorded is the $^{15}N\{^{1}H\}$-NOE. Even in the absence of assignments this experiment will immediately reveal whether flexible tails or long flexible loops are present and thereby help to eliminate or truncate such flexible moieties.

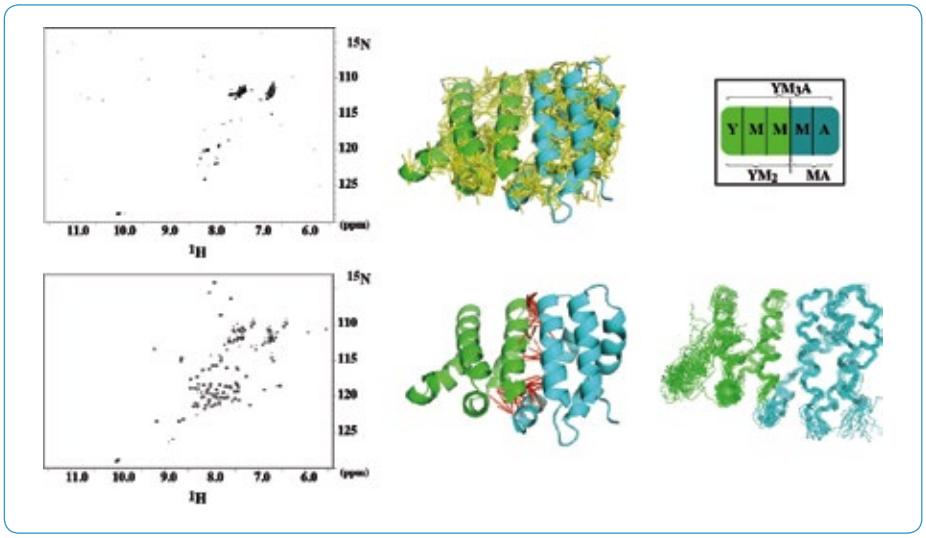


Fig. 3: Protein structure determination. Left ^{1}H-^{15}N correlation spectra of the N-terminal YM_2 fragment in absence (top) or presence (bottom) of the unlabeled C-terminal fragment. Center Non-interfacial (top) and interfacial (bottom) medium- and long-range NOEs. Right Conformers of the protein complex and a scheme of the YM_3A protein.

Another question of interest often is whether the protein interacts with a small molecule, a typical question in drug-discovery [15,16]. In contrast to biochemical assays only two components exist in the NMR experiment, and if pH, salt content and temperature are tightly controlled, no false-positive will be seen. In the protein-observe methods the protein is usually ^{15}N labeled. When adding the small molecule, peaks from residues in contact with the ligand will shift, indicating that the small molecule binds to the protein. If assignments already exist, the binding site can be rapidly identified. The advantage of the ligand-observe techniques is that no labeled protein is required. The saturation-transfer difference experiment (STD) [17] has become popular to detect binding on the ligand. The experiment is very simple to perform, and it works very well in presence of large receptor proteins.

Prof. Roland Sigel here at UZH determines interactions of metals with RNA as parts of the so-called riboswitches. The structural biology of RNA is much more versatile and interesting than DNA. Moreover, RNA can be labeled at comparably low cost. The determination of RNA structure has become routine nowadays [18], and procedures for structure determination are very similar to those used for proteins.

Another field from the life science area that has attracted some attention is metabolomics [19]. In metabolomics the presence of metabolites in biological fluids, e.g. in urine, is monitored. Urine from animals living under a certain diet can be compared to standard conditions. Metabolites can then be identified from reference spectra or data bases. Only soluble metabolites in significant concentrations can be detected.

Summary

This is a collection of a few applications that are frequently used in the Chemistry Department at UZH, and this overview is far from complete. Interested readers are therefore referred to the literature or to the book mentioned above [1].

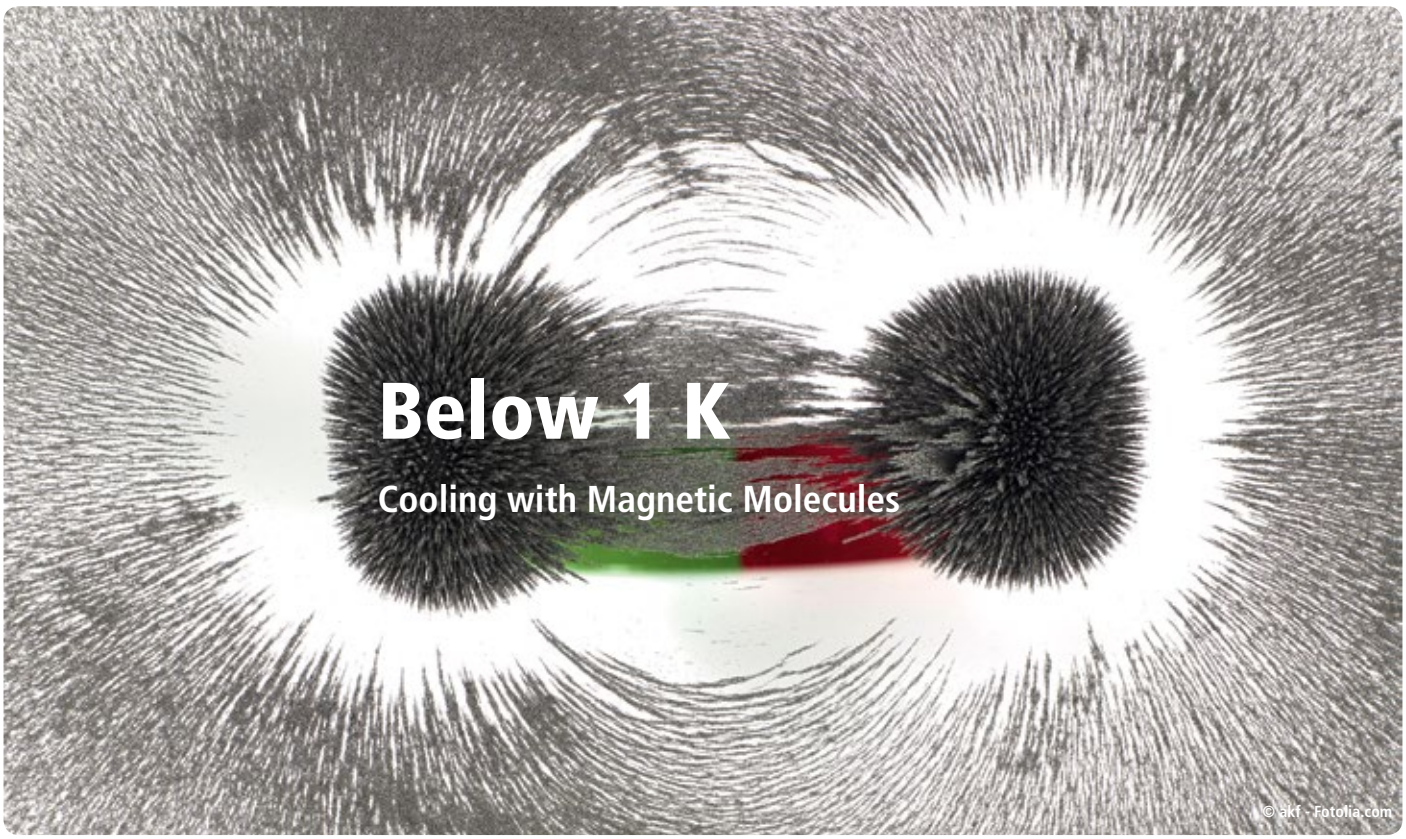
References:

[1] Jurt, S. & Zerbe, O. NMR for Chemists and Life Scientists (VCH-Wiley, Weinheim) (2013).
[2] Keeler J. *et al.:* Meth. Enzymol. 239, 145-207 (1994)
[3] Hyberts S. G. *et al.:* Top Curr. Chem. 316, 125-148´ (2012)
[4] Schanda P. *et al.:* J. Biomol. NMR 33, 199-211 (2005)
[5] Schanda P. and Brutscher B.: J. Am. Chem. Soc. 127, 8014-8015 (2005)
[6] Russell D. J. *et al.:* J. Nat. Prod. 63, 1047-1049 (2000)
[7] Johnson C. S.: Prog. NMR Spectrosc. 34, 203-256 (1999)
[8] Bain A. D.: Progr. NMR Spectrosc. 43, 63-103 (2003)
[9] Wider G.: Methods Enzymol. 394, 382-398 (2005)
[10] LeMaster D. M.: Methods Enzymol. 177, 23-43 (1989)

Please ask the authors for further references.

Authors: Simon Jurt, Oliver Zerbe
Department of Chemistry, University of Zurich, Switzerland

Contact
Oliver Zerbe
Department of Chemistry
University of Zurich
Zurich, Switzerland
oliver.zerbe@chem.uzh.ch



An international team of scientists from Bielefeld, Manchester and Zaragoza achieved the first sub-Kelvin cooling with magnetic molecules. The results demonstrate that it is indeed possible to use magnetic molecules for magnetic refrigeration at such low temperatures, but moreover it also shows that the respective thermodynamic processes are very different from those using paramagnets. Magnetic molecules offer the opportunity to design the important curves of constant entropy (isentropes) through the design of appropriate magnetic molecules.

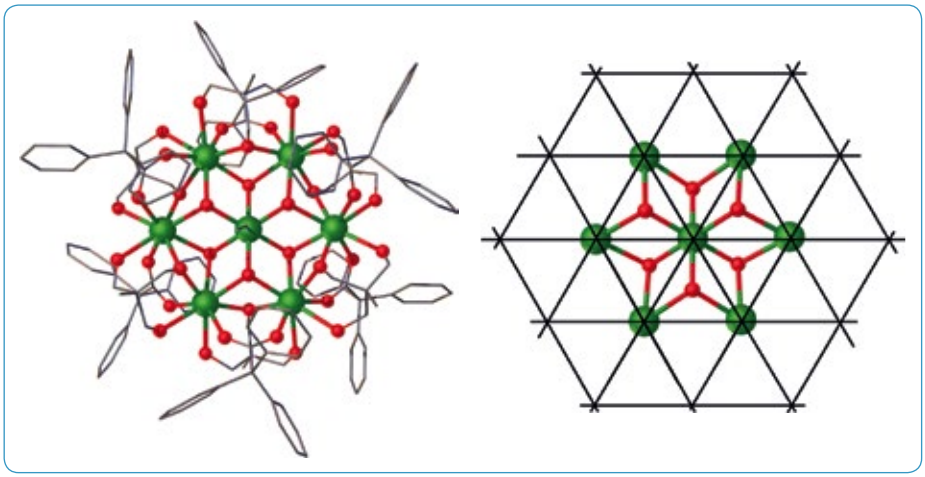


Fig. 1: The structure of $[Gd_7(OH)_6(thmeH_2)_5(thmeH)(tpa)_6(MeCN)_2](NO_3)_2$ ["Gd_7"; H_3thme = tris(hydroxymethyl)ethane; Htpa = triphenylacetic acid] (l.h.s.) is a finite size cutout of a triangular lattice.

The Magnetocaloric Effect

That magnetic materials can change their temperature is known since the pioneering work of Emil Warburg in 1881. He discovered that plain iron changes its temperature when an applied magnetic field is removed. This magnetocaloric effect (MCE) can be used for a variety of cooling applications, for instance in special room-temperature refrigerators that work without refrigerant fluids. The magnetocaloric effect is also employed in order to achieve the lowest possible temperatures in thermodynamic cycles such as the Carnot or Ericsson cycles, which work with paramagnetic substances. Sub-Kelvin temperatures were experimentally obtained already in 1933 by W.F. Giauque who received the Nobel prize for his achievement in 1949. In contrast to paramagnets, magnetic molecules should offer more flexibility concerning the cooling process, but until now this remained a hypothetical option.

Cooling Below 1 K

The authors of [1] achieved a cooling down to approximately 0.2 Kelvin using a substance that consists of magnetic molecules, i.e. tiny nanomagnets. For their experiment they employed the molecular cluster $[Gd_7(OH)_6(thmeH_2)_5(thmeH)(tpa)_6(MeCN)_2](NO_3)_2$ ["Gd_7"; H_3thme = tris(hydroxymethyl)ethane; Htpa = triphenylacetic acid] which consists of a planar centered hexagon of weakly AF coupled Gd(III) ions, each of which has an electronic spin s = 7/2. Figure 1

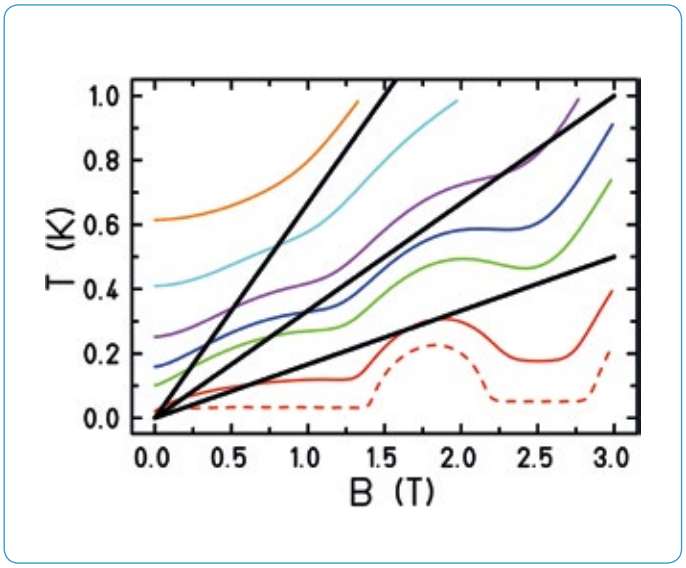


Fig. 2: Theoretical curves of constant entropy, i.e. isentropes of Gd_7 (coloured) compared to those of a paramagnet (straight black lines).

shows the molecular structure which is related to the well-known triangular lattice antiferromagnet. Besides the fact that this investigation constitutes the first sub-Kelvin cooling experiment with magnetic molecules it also exemplifies the richness of adiabatic processes in interacting magnetic quantum systems.

For a paramagnet the curves of constant entropy, on which the important adiabatic processes run up or down, are straight lines heading towards the origin of the T-B plane (compare black lines in Fig. 2). Their slope, which is the cooling rate, is always T/B for each pair of temperature T and field B, from where one wants to cool down, for instance. Magnetic molecules allow one to achieve very different cooling rates in certain parts of the T-B plane, especially close to the B axis. Such a behavior could be theoretically predicted for Gd_7 and successfully measured. The bumpy structure of the isentropes of Gd_7 shown in Figure 2 as colored curves reflects the unusual structure of magnetic energy levels present in Gd_7 and simultaneously demonstrates that an interacting quantum magnet may produce cooling rates that are much larger in certain T-B regions compared to a paramagnet, a phenomenon that is termed enhanced magnetocaloric effect. In addition, processes such as heating upon decreasing the field are possible, too, which never happen with paramagnets.

Frustrated States

The unusual and pronounced bumpy structure of the isentropes of Gd_7 (Fig. 2), which could be followed in the cooling experiments, is an outcome of the frustrated nature of the antiferromagnetic interactions in Gd_7. Since the centered hexagon consists of triangles, the antiferromagnetic interaction is unable to constitute a magnetic ground state of pairwise "happy" combinations of up and down spins. Such a situation is termed "frustrated" [2]. Frustration often leads to an unusual bunching of low-lying energy levels, which as a function of applied magnetic field may vary strongly. It is this strong variation of the density of low-lying energy levels that produces the beautiful bumpy entropy landscape. The hope is thus that in the future we would be able to design isentropes according to our needs by means of rational design of magnetic molecules.

References

[1] Sharples J. W. *et al.:* Nature Communications 5, 5321 (2014)
[2] Schnack J.: Dalton Trans. 39, 4677-4686 (2010)

Contact

Jürgen Schnack
Faculty of Physics
University of Bielefeld
Bielefeld, Germany
jschnack@uni-bielefeld.de

Nucleophile Selective Cross-Coupling Reactions on Aromatic Rings

A New Tool in the Synthetic Chemist's Tool Box

Nucleophile selective cross-coupling reactions on aromatic rings can differentiate between different nucleophilic functional groups: This can imply different metals such as tin and boron, where a Stille cross-coupling can take place first without any reaction of the boron centre. More challengingly, nucleophilic sites that contain the same metal, but in slightly different chemical environments can also undergo selective cross-coupling reactions. Emerging applications for such cross-coupling reactions in materials science are discussed.

Introduction

Modern organic synthesis is unthinkable without cross-coupling reactions. The most eminent ones are perhaps the Stille-reaction, where organostannanes serve as nucleophilic component, the Suzuki-reaction where organo boron reagents are coupled, or the Negishi-reaction which employs organo zinc compounds. With a desire to build more and more complex molecules in shorter and more efficient ways, ideally without protection groups, the selectivity of such reactions is now the focus of research. It is well established that the reactivity of the electrophilic component, R-X, highly depends on the nature of X; an iodo-substituent is more reactive than a bromo- or a chlorine substituent in nearly all cases. Unsurprisingly, electrophile-selective reactions are now well known. Nucleophile selective reactions on the other hand are still very rare, although they open very efficient access routes to materials that were not conceivable before.

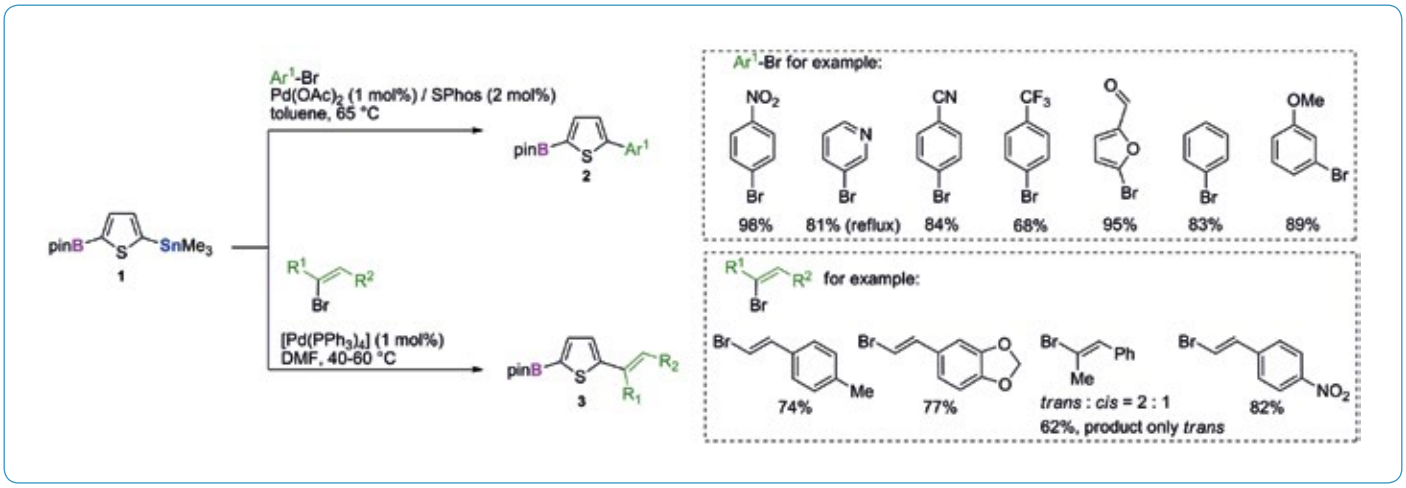
Selective Cross-Coupling of Aromatic Dinucleophiles with Different Metals

Aromatic reagents bearing two different nucleophilic sites, i.e. different metal or hemi-metal functional groups, are exceedingly rare. In 1989, the first such reagent was reported, a benzene containing a stannyl group and a boronic ester [1]. Although it was used in a Stille selective cross-coupling reaction, no general use was demonstrated. In 2012, thiophene 1 with both a trialkyl-tin and a boronic ester functional group in chemically equivalent positions was developed (Scheme 1) [2]. By careful optimisation of the reaction conditions, a high yielding and completely selective Stille coupling reaction could be developed that took place in the presence of a boronic ester on the same molecule. Those conditions were applicable for a panoply of different aromatic electrophiles. After the completion of the Stille reaction, adding a base, water and a second electrophile to the reaction mixture, Suzuki-reactions could be carried out, also in high yields.

However, not only arylhalides and pseudohalides are suitable electrophilic reaction partners; vinyl- and alkynylbromides have also been successfully employed in this type of nucleophile selective cross-coupling reactions (Scheme 1) [3].

This new synthetic tool could also be successfully expanded to a reaction that was both nucleophile- and electrophile selective [4]. With the same di-nucleophilic thiophene 1, di- and even trielectrophiles bearing iodo- bromo- or trifluorosulfonyl-groups could be used as cross-coupling partners.

First applications for the products of such nucleophile- and electrophile selective reactions are emerging in materials science: The cross-coupling reaction of different heterocycles that leads to products which still contain a metal functional group and a halide are potential monomers for the synthesis of regio-



Scheme 1: Stille-selective cross-coupling reactions with aryl- and vinyl bromides. The boronic ester stays in place and can be further used in Suzuki cross-coupling reactions.

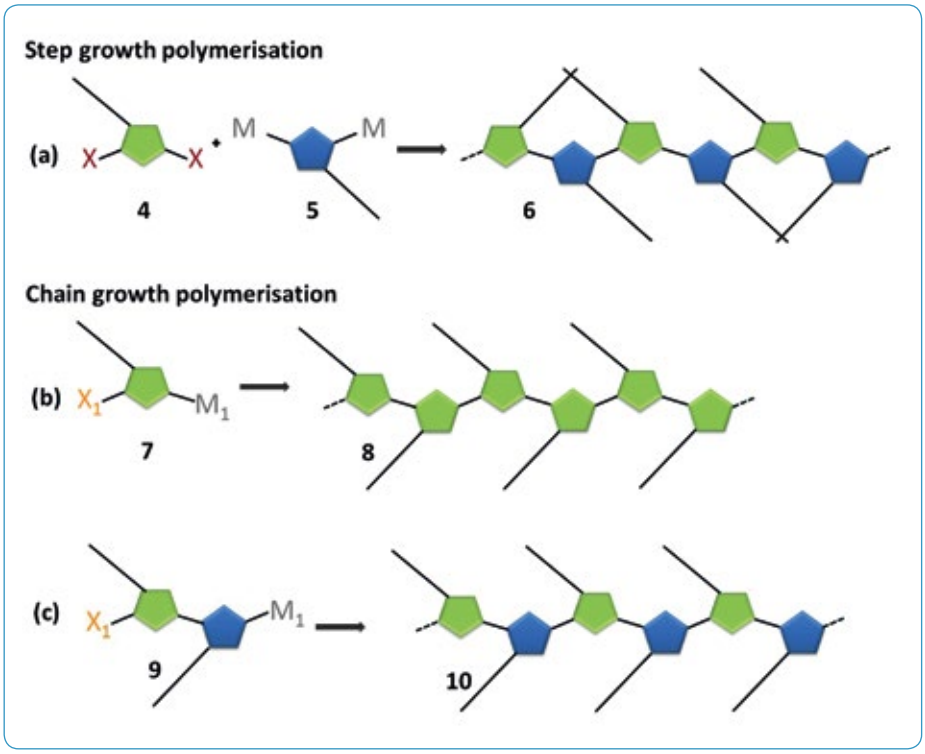


Fig. 1: Two possible pathways for the preparation of semiconducting polymers.

regular semiconducting polymers. In such polymers, the combination of different heterocycles is a much used tool for tuning the band gap of the material. The most common synthetic route is a step growth polycondensation polymerisation, where two monomers, X–Monomer[1]–X and M–Monomer[2]–M, 4 and 5, are cross-coupled (Figure 1). While this process is conceptionally simple, the process does not always work well in practice: Often, only short oligomers can be obtained. Moreover, if the monomers are unsymmetric, solubilising side chains may be regioirregular, leading to torsional defects. An alternative is the chain growth of monomers of type 7, which by their very design should only give regioregular polymers. A compound such as 9a (Figure 1) would combine the advantages of both approaches. As a test monomer, compound 9a (Scheme 2) has been synthesised in a nucleophile and electrophile selective cross-coupling reaction. Initial results for the polymerisation are promising: under Suzuki conditions, a red polymer is formed within a few minutes.

Selective Cross-Coupling of Differentiating Between the Same Metal Atoms at Different Sites

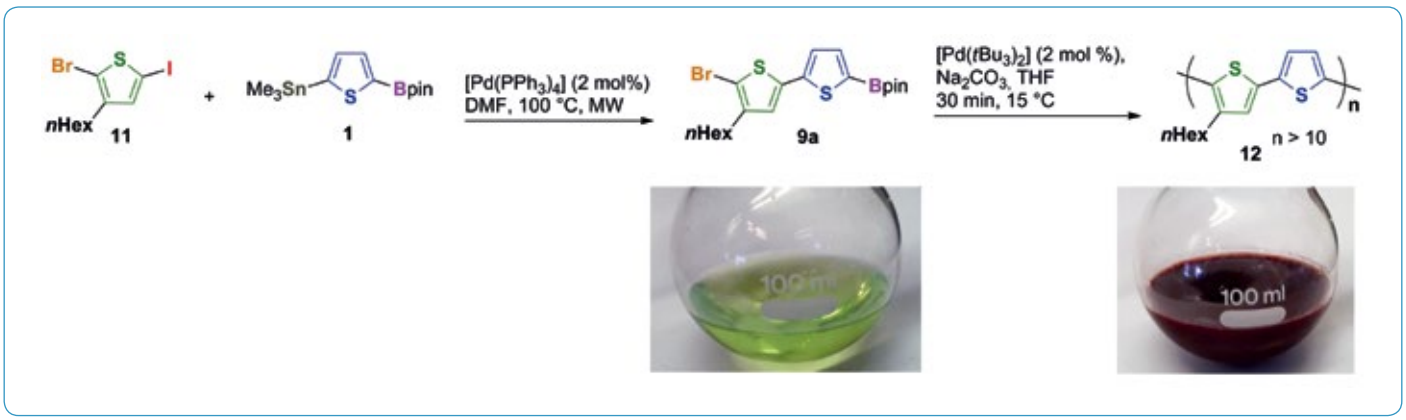
Cross-coupling reactions using organometallic compounds that contain the same metal in different chemical environments, with only one of these sites selectively reacting, are very difficult to perform and therefore exceedingly rare. One such example was demonstrated recently by Pengfei Li and co-workers [5]. Although this level of selectivity is impressive, it is conceivable to prepare the products obtained after the first or second cross-coupling step via other synthetic routes.

A more difficult problem is presented if one of the metal functional groups is to remain in the material. We recently wished to prepare a stannole containing semiconducting polymer. Such materials had not been reported before, but for low molecular weight stannoles, it has been discovered that due to a pronounced σ*-π* conjugation effect, the HOMO-LUMO gap is much reduced [6]. Therefore, a well processable stannole containing polymer could be an interesting material for organic semiconductor applications such as solar cells.

In a stannole, the tin atom can be viewed as a vinyl-tin species, which readily undergo Stille cross-coupling reactions. Furthermore, it has been demonstrated recently that the related stanna-fluorenes also react readily with 1,2-dibromobenzenes in a palladium catalysed Stille reaction to give triphenylenes (Scheme 3) [7].

In our laboratories, we used the concept of kinetic protection to differentiate between the tin centre of a stannole and exocyclic trimethyltin-substituents. We envisioned the preparation of a stannole containing polymer 16 using a stannole monomer that was flanked by iodinated thiophenes 17 (Scheme 4) [8]. The other monomer therefore needed to be a thiophene with two metal functional groups to be able to synthesise the polymer in a palladium catalysed polycondensation. In polycondensation reactions, high molecular weights can only be obtained if the conversion of the reaction is very high and the stoichiometry of the reagents is very accurate. In other words, even a small amount of side reactions will prevent the polymer from forming. Initial results indicated that small groups such as methyl and *n*-butyl on the stannole tin atom were attacked under Stille cross-coupling reactions and therefore unsuitable.

However, when the R^1 group on the tin atom in the stannole is phenyl, then this site is kinetically completely protected. Under Stille reaction conditions, the polymer formed in a high yield of 90%. It was a dark purple polymer which showed a substantial bathochromic shift of its absorption maximum (λ_{max} = 536 nm in chloroform, λ_{max} = 585 nm as a film, Figure 2) compared to regioregular poly(hexylthiophene) (λ_{max} = 449 nm). The polymer had a number average molecular weight of M_n = 6.8 kDa and a weight average weight of M_W = 17.0 kDa (gel perme-



Scheme 2: Preparation of monomer 9a by nucleophile- and electrophile selective Stille cross-coupling reaction and subsequent polymerisation by Suzuki reaction.

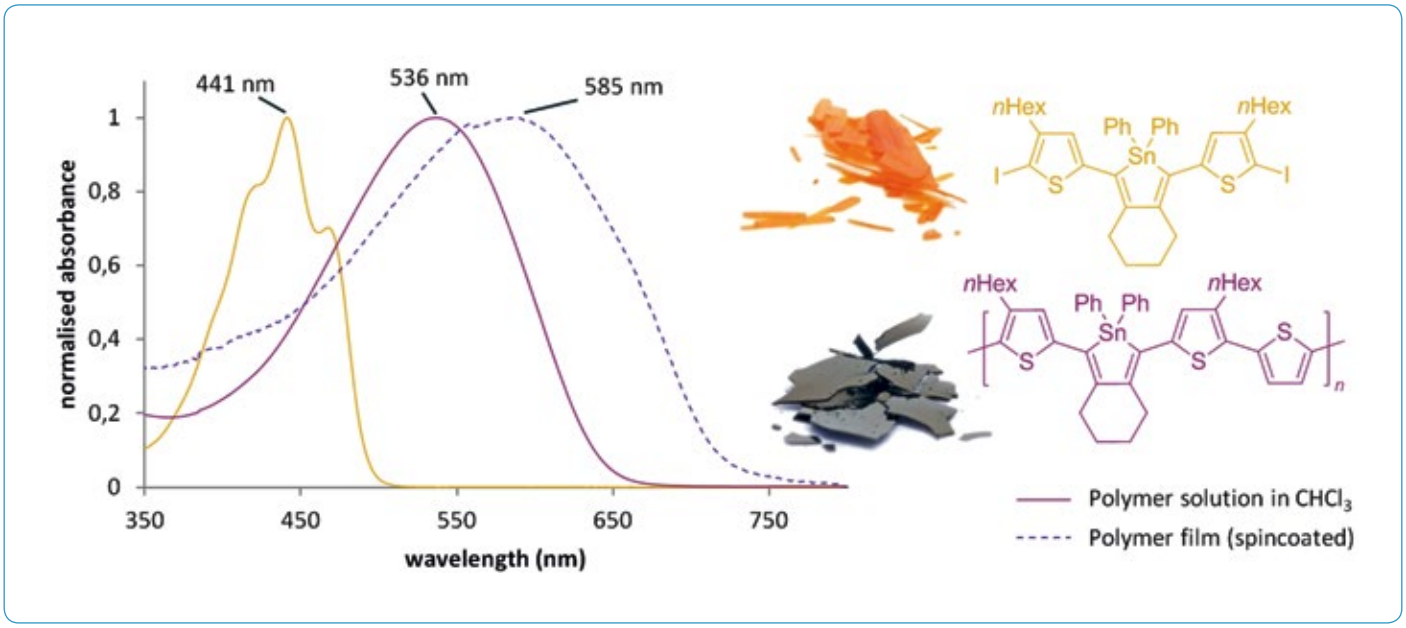


Fig. 2: UV-vis spectra of the monomer (yellow) and the stannole containing polymer (purple; Eg (film) = 1.7 eV).

ation chromatography, calibrated against polystyrene). Therefore, the nucleophile selectivity must have been extraordinary high.

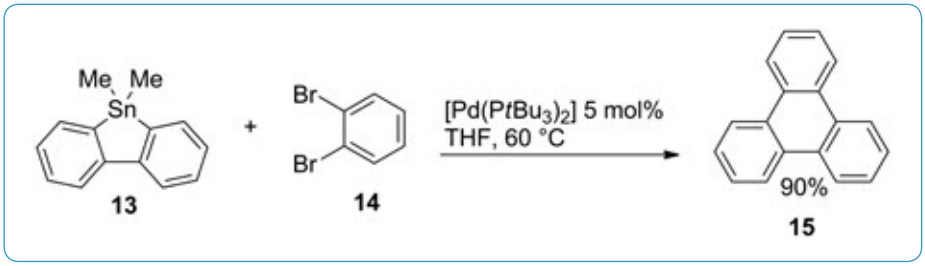
Conclusions

Nucleophile selective cross-coupling reactions of aromatic compounds promise to be a fruitful field of research. First successful results have been obtained, differentiating between nucleophiles demanding different reaction conditions such as organo-tin substituents and organo-boron compounds. In addition, nucleophile selective cross-coupling reactions that can even differentiate between functional groups containing the same (semi)metal, but in slightly different chemical environments are now possible. That such reactions lend access to hitherto unknown materials such as stannole containing semiconducting polymers demonstrates their synthetic prowess: Aromatic dinucleophilic reagents will prove a powerful tool in the convergent, protection group free syntheses of complex natural products and materials, where both nucleophilic sites can be introduced early into a molecular fragment.

References

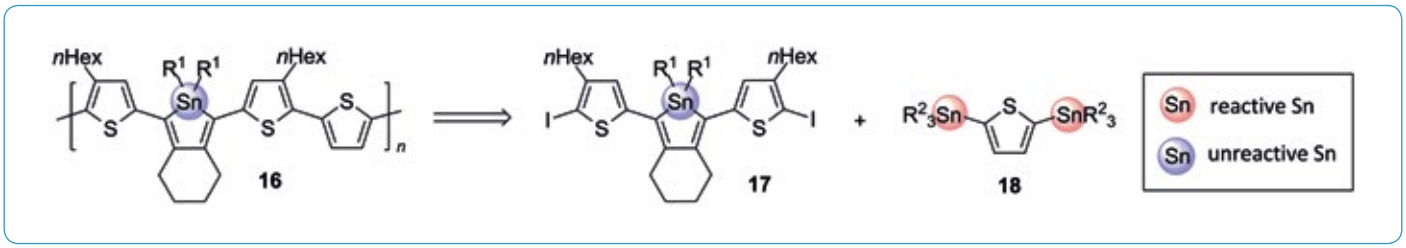
[1] Yamamoto Y. *et al.:* J. Org. Chem. 54, 4736, (1989)
[2] Linshoeft J. *et al.:* Org. Lett. 14, 5644 (2012)
[3] He L.-Y. *et al.:* Eur. J. Org. Chem., DOI 10.1002/ejoc.201500138 [EMID:d07884f3f3907cc6]
[4] Heinrich A. C. J. *et al.:* Org. Lett. , 15, 4666 (2013)
[5] Xu L. *et al.:* Angew. Chem. Int. Ed. 53, 1822 (2014)
[6] Yamaguchi S. *et al.:* Organometallics, 17, 4910, (1998)
[7] a) Nagao I. *et al.:* Angew. Chem. Int. Ed. , 48, 7573 (2009); b) Kumar B. *et al.:* J. Org. Chem., 77, 311 (2012); c) A. Kamimura *et al.:* Org. Lett., 15, 1402 (2013)
[8] a) Linshoeft J. *et al.:* Acta Cryst., E70, o1133 (2014); b) Linshoeft J. *et al.:* Angew. Chem. Int. Ed. 53, 12916 (2014)

Authors
A. Staubitz, J. Linshoeft, A. C. J. Heinrich, University of Kiel, Germany



Scheme 3: Example of a facile reaction of stannafluorene with 1,2-dibromobenzene under palladium catalysis.

Contact
Prof. Dr. Anne Staubitz
Otto-Diels-Institute for Organic Chemistry
University of Kiel, Germany
www.otto-diels-institut.de/staubitz/
astaubitz@oc.uni-kiel.de



Scheme 4: Retrosynthetic analysis of a stannole containing polymer.

Capacitive Deionization

Energy Efficient Water Desalination

Capacitive deionization is an emerging technology for energy efficient water treatment. Having originated from first concept studies in 1960, today's commercial CDI systems target water desalination application ranging from producing potable water from brackish water to the remediation of industrial or mining waste water.

What is Capacitive Deionization?

Capacitive Deionization (CDI) is an emerging technology that can be used for energy efficient removal of dissolved, charged species from water – for example for desalination of brackish or sea water. Yet, CDI is much more than an attractive tool to generate potable water and has been applied also to wastewater remediation and water softening. The high energy efficiency of CDI for the desalination of water with a low salt concentration (typically below 10 g/l) is due to the fact that the salt ions, which are the minority component in the water, are removed from the mixture. By contrast, conventional water desalination systems (such as reverse osmosis, and distillation) instead remove the majority component, the water molecules, from salty feedwater. CDI is typically characterized by an intermittent operation between ion electrosorption (until the electrodes are fully saturated / fully charged) and electrode regeneration (which relates to discharging the electrodes to release the electrosorbed ions). Considering a full CDI cycle, the invested charge for ion removal can be largely recovered during electrode regeneration, enabling energy consumption significantly below 1 kWh/m^3 for desalinated brackish water. As a result of the intermittent operation, sequential ion-depleted and ion-enriched stream are generated, yielding a water recovery which can be significantly above 50%, and as high as 90%. Water recovery is an important performance metric for desalination technologies and is defined as the ratio of freshwater volume over inlet volume.

CDI Theory: Ion Electrosorption via Double-Layer Formation

Ion electrosorption is an often encountered phenomenon in nature and technology; it describes the electrostatically induced adsorption of ions or electrically charged molecules at the interface of an electrode surface with opposite charge (Fig. 1A). The high reversibility and energy efficiency of this process is exploited in the operation of high power density and long-lasting electrical double-layer capacitors, also known as supercapacitors. Instead of just exploiting efficient storage and recovery of electrical charges, the process of electrosorption can also be seen from the point of view of the water: when ions are electrosorbed, the formation of an electrical double-layer effectively immobilizes the ions at the fluid-solid interface. Thus, electrosorption can also be used to remove ionic species from aqueous media, in applications such as water desalination, water softening, and

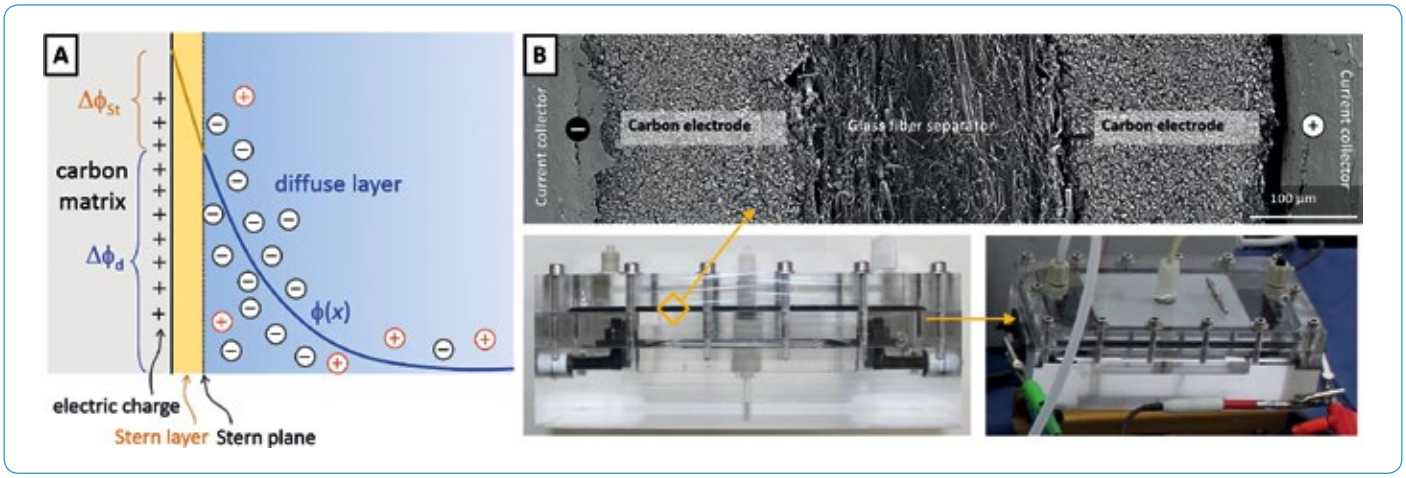


Fig. 1: (A) Electrical double-layer formation at a planar solid-liquid interface between an electrically charged electrode and water with dissolved ions. (B) Scanning electron micrograph cross-section of a pair of electrodes composed of activated carbon and polymer binder, put on graphite foil current collector and separated by a glass fiber separator. This pair of electrodes is part of a multiple stacking inside a CDI laboratory cell (visible inside the optically transparent cell) which is then used for water desalination applications.

wastewater treatment. The structural details of the nanoscale electric double-layer are crucial to electrosorption performance, and determining this structure forms a highly active area of research. Further understanding in this area promises to form the basis for future breakthroughs in electrosorption technologies, such as CDI.

Materials and Setups

The basic element of any CDI cell is a single pair of carbon electrodes (Fig. 1B); CDI systems employ such electrodes with various sizes, thicknesses, and a certain number of pairs. Commonly, activated carbons are used as the active component that affords ion electrosorption by exhibiting a large specific surface area (typically 1200 – 1500 m^2/g) and specific pore volume (around 0.8 – 1.0 cm^3/g). Polymer binders, such as polyvinylidenfluoride or polytetrafluorethylene, are admixed to the activated carbon powder (5 – 10 wt%) to finally obtain 100 – 250 µm thick film electrodes. To avoid electro-corrosion, such electrodes are commonly applied to graphite foils which effectively acts as a current collector. Finally, between the electrodes, a porous separator (typically glass fiber) is placed to ensure electrical isolation and facile flow of the aqueous solution by the porous carbon materials. The performance of CDI electrodes has been studied intensively, and the ability of the electrodes to remove salt from the feedwater has tripled over the past decade, from roughly 5 mg_{NaCl}, stored/g_{carbon} to 15 $mg_{NaCl,\ stored}/g_{carbon}$. In addition to their ability to remove salt, electrodes have also significantly improved in the rate of salt removal, achieving up to 2.5 $mg_{NaCl,\ stored}/g_{carbon}$/min. We have not reached fundamental limits in both the salt removal or rate capabilities of CDI electrodes, and so further breakthroughs can be achieved in the coming years.

CDI cells can be constructed in various architectures (Fig. 2). As ions need to be removed from the in-fed water stream, it is highly desirable to maximize the interaction volume between solution and electrode pores, and a flow-through setup is perhaps the most direct way to achieve this feat. In such a cell, the electrode is constructed from a material with open porosity (such as carbon aerogel) and the saline water flows perpendicular to the electrodes and through the electrode stack. A more conventional setup with flow between electrodes has the benefits of dense current collectors and more facile water flow between the electrodes. Such a flow between setup was the original CDI design in the 1960s and is still today the standard geometry used in commercial devices.

The efficiency and salt sorption capacity of CDI cells can be significantly improved by placing ion exchange membranes in front of the carbon composite electrodes. To understand why membranes allow for performance enhancements, we have to briefly revisit the concept of ion electrosorption. CDI only affords the removal of ions by electrosorption counter-balancing an electrical charge at the electrode. Yet, this process of electric charge compensation can be accomplished, in theory, in three ways: either by counter-ion adsorption, co-ion expulsion, or a combination thereof, namely ion swapping (Fig. 3). Counter-ions have a charge opposite to the electric charge at the electrode and their preferential electrosorption effectively depletes the feedwater of salt ions. Charge accommodation can also be accomplished by expulsion of co-ions, or ions with the same charge sign as the electrode, a process which increases the salt concentration in the feedwater flow. It is important to consider the contribution of both effects when evaluating the salt sorption capacity. The last ideal case, pure co-ion desorption, will not occur under normal operation, rather CDI is typically characterized by some co-ion desorption and significantly more counterion adsorption. The main benefit of adding membranes to the CDI cell is the reduction of the detrimental effect of co-ion expulsion, as the membranes effectively block co-ions from carrying parasitic current, and can thus increase the salt storage capacity of the electrode.

A new Trend in CDI: Flow Electrode

Very recently, a new architectural class for CDI was demonstrated employing carbon flow electrodes which can be pumped through electrode compartments; the latter are separated from the feed water stream typically by ion exchange

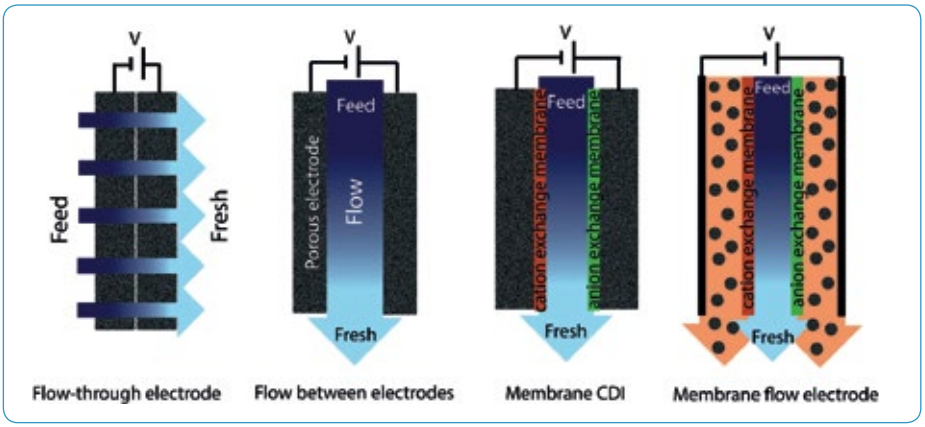


Fig. 2: CDI architectures: flow-through CDI, flow between CDI, flow between membrane CDI, and membrane flow electrode CDI.

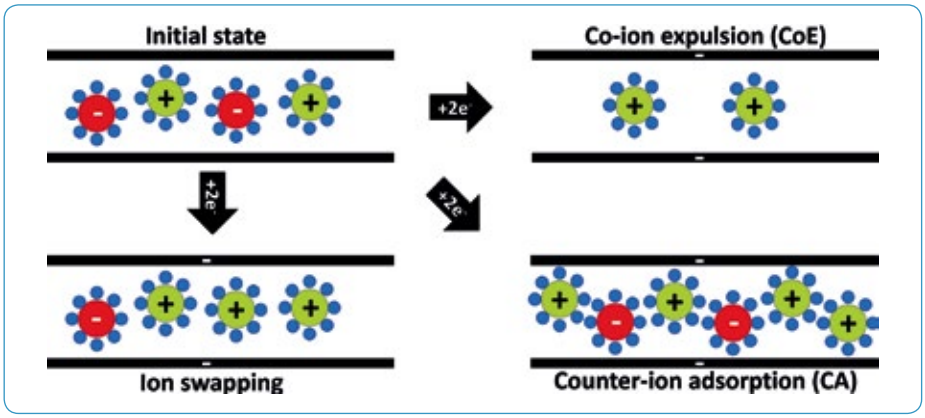


Fig. 3: Three different basic concepts for charge compensation via ion electrosorption.

membranes, although also porous separator configurations are possible. Such a flowable setup has several advantages. First, the feed water flowing through a single cell can be continuously desalinated, as the discharge of the active carbon particles (regeneration) can occur as a separate process downstream of the cell. In contrast, in all previous CDI architectures based on static electrodes, the cell can only desalinate for a limited period of time until the EDLs of the porous electrodes have been fully charged, and then desalination must cease while the cell is discharged to enable subsequent desalination cycles. This intermittent operation also can require complicated fluidic handling as desalinated stream (during charging) and brine streams (during discharging) emerge, at different times, from the same spacer between the electrodes. A second major benefit is that FCDI, by continuously introducing uncharged carbon particles into the charging cell, can effectively increase the capacitance available for desalination above that of static electrode CDI systems. Thus, FCDI can desalinate higher salinity streams than static CDI systems, and the complete desalination of high salinity feeds, such as 32 g/l salt concentration, roughly that of sea water, has been reported. The desalination of sea water was previously not practically attainable by static electrode CDI systems, and thus flow electrode systems are an exciting advance.

Conclusions and Outlook

The field of capacitive deionization has seen a tremendous growth over the last 5 years with an increasing number of articles being published, a conference series on the topic has been established (2015 Saarbrücken, Germany; 2017 Haifa, Israel), and an international working group on the topic (www.cdi-electrosorption.org) which began its work in 2014. The exponentially increasing interest in CDI is motivated by several unique advantages. In contrast to many conventional desalination techniques, CDI operates at low pressures (i.e., sub-osmotic), at room temperature, and requires only small applied voltages (typically 1 V per one pair of electrodes). Thus, the technology does not require high pressure pumps or heat sources. In addition to exhibiting low amounts of energy for salt removal, a large fraction of the invested charge is recovered during electrode regeneration by simply, without chemical agents, discharging the cell.

Contact

Jun.-Prof. Dr. Volker Presser
INM – Leibniz-Institut für Neue Materialien GmbH
Saarbrücken, volker.presser@inm-gmbh.de

Prof. Matthew E. Suss
Faculty of Mechanical Engineering
Technion - Israel Institute of Technology
Haifa, Israel"

X-ray Free Electron Lasers

A New Revolution in Structural Studies

X-ray crystallography is a crucial research technique for chemists who would like to investigate the structure of their compounds in solid state.

So far lots of Nobel prizes have been awarded for research fundamental to or applying crystallographic methods, from the early discoveries of Röntgen until the recent prizes for research on quasicrystals or implementation of theoretical methods in the refinement of macromolecules. Nevertheless, the field still undergoes a rapid development and more breakthroughs are expected, in particular in the field of X-ray free electron laser (XFEL) crystallography.

X-ray Free Electron Lasers

X-ray free electron lasers are devices that produce very short (in order of femtoseconds) pulses of very coherent X-rays [1]. This could bring so far unavailable possibilities in structural research, providing access to time-resolved studies, following reactions during the diffraction experiment or overcoming the problem of radiation damage.

So far there are two facilities offering access to X-ray free electron lasers suitable for high-resolution structural studies, although there are many institutes with operating free electron lasers. These are LCLS (Linac Coherent Light Source) in Stanford and Spring-8 (Super Photon Ring) SACLA in Japan. A European facility will be open for users in 2017 in DESY (Hamburg), also a Swiss source is planned for 2016 [2].

Production of X-Ray Flashes

How do the X-ray free electron lasers produce their X-ray pulses? First bunches of electrons have to be accelerated which takes place in very long resonators where the oscillating microwaves inside transfer energy to the electrons. Each stage of this procedure is controlled very strictly in order to ensure a very well-defined regular electron beam. Then the electrons are directed to the so-called undulator which is an array of magnets with alternating north and south poles (Fig. 1) [3]. This undulator wiggles the movement of electrons. As a result they start emitting X-rays. X-rays are faster than electrons – due to their interaction the electrons form assemblies resembling "disks" which emit very coherent radiation – this is how X-ray flashes are produced. This phenomenon is called self-amplified spontaneous emission. The X-ray flashes are released, the electrons are diverted at the end so that they do not come out together with the flashes.

The produced beams have unique characteristics. They are much brighter than radiation produced by the most recent generation of synchrotrons. Their wavelength can be tuned from 10 to 0.5 Å. The pulses are very short, even at 1 fs, and energetic; they could cut through steel.

But they could be also used for structure determination. It was suspected that this could be done even before XFELs were constructed. Simulations were done for protein molecules, such as the model protein in crystallography, the lysozyme [4] showing that before the molecule is destroyed as a result of Coulomb expansion, it diffracts and the diffracted beams can be detected. Now the first XFEL was open in 2009 and the first protein structure determination with the aid of XFELs was performed in 2011 for the membrane protein Photosystem I, proving that the principle of "diffraction before destruction" can really be implemented [5].

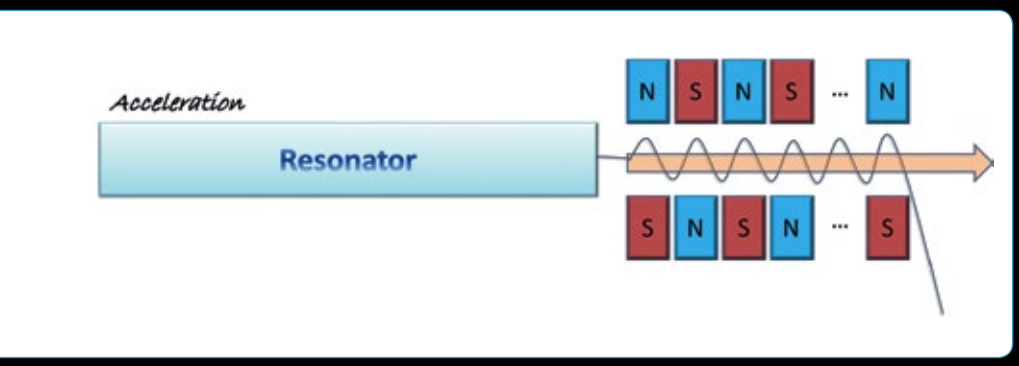


Fig. 1: The principle of formation of X-ray pulses in XFELs (see text)

Sample Preparation

So far there are two main options for sample delivery in XFELs (Fig. 2). In the first option nanocrystals are suspended in a liquid or gel matrix and continuously injected into the place where they are hit by X-ray pulses. Before destruction they diffract and the diffracted beams

are detected by the detector. This is the basis of serial femtosecond crystallography where changes in the sample, for instance upon irradiation, can be monitored in a time-resolved manner. There is no control of the crystals orientation or quality.

Another approach is to use a fixed target, such as a large crystal or microcrystals embedded in a film. Different places in such a collective sample are probed with X-ray pulses, therefore the sample holder has to be moved so that the places can be chosen.

Data Collection & Analysis

The detectors in XFEL crystallography, e. g. CSPAD (silicon pixel array detector), must meet specific challenges which are the high radiation intensities and exposures at a femtosecond-time-scale at a rate of hundreds of pulses per second [6].

Data integration is problematic as the diffraction data are collected for many nanocrystals in random orientations. Additional source of experimental error are the instabilities in the pulse intensity and the noise from accompanying solvent. Therefore during data integration a Monte Carlo approach is adopted, assuming that these orientations are perfectly random. But first the data need to be indexed – this is done selecting reflections which nearly fulfill the Bragg's condition [7].

Already a couple of alternative software suites are available for treatment of these data, such as "Cheetah" or "Crystfel" [8]. Lots of data are produced, in the amounts of hundreds of terabytes. Not all of these data are useful, so they have to be analyzed and the useful data must be selected. This is now called the "hit finding" and is a very time-consuming procedure. Typically out of about 4 millions of collected frames 400 000 are useful and 300 000 can be indexed [8].

Outlook

Nevertheless, in spite of these problems XFEL crystallography opens new pathways for answering of still unanswered questions, concerning e. g. the structure of S-states in the Kok cycle of Photosystem II or imaging of single virus particles under physiological conditions. Some of the recent breakthroughs include obtaining radiation-damage-free structure of PSII WOC which has important implications for the understanding of the natural photosynthesis process and design of catalysts for artificial photosynthesis [9].

Acknowledgement

The author gratefully acknowledges Prof. Dr. Stefanie Dehnen and Dr. Klaus Harms (generous support and helpful discussions).

References

[1] Spence J. C. H. *et al.*: Rep. Prog. Phys. 75, 102601. (2012)
[2] www.xfel.eu
[3] Waldrop M. M.: Nature 505, 606 (2014)
[4] Neutze R. *et al.*: Nature 2 406, 752 (2000)
[5] Chapman H. N. *et al.*: Nature 2011, 470, 73.
[6] Herrmann S. *et al.*: Nucl. Instr. and Meth. in Phys. Res. A718, 550 (2013)
[7] Foucar L. *et al.*: Comp. Phys. Commun. 183, 2207 (2012)
[8] Barty A. *et al.*: J. Appl. Cryst. 47, 1118 (2014)
[9] (a) Kupitz C. *et al.*: Nature, 513, 261, (2014) (b) Kern J. *et al.*: Nature Commun. 5, 4371, (2014) (c) Suga M. *et al.*: Nature 517, 99 (2015)

Contact

Dr. Malgorzata Holynska
Department of Chemistry
Philipps University Marburg
Marburg, Germany
holynska@staff.uni-marburg.de

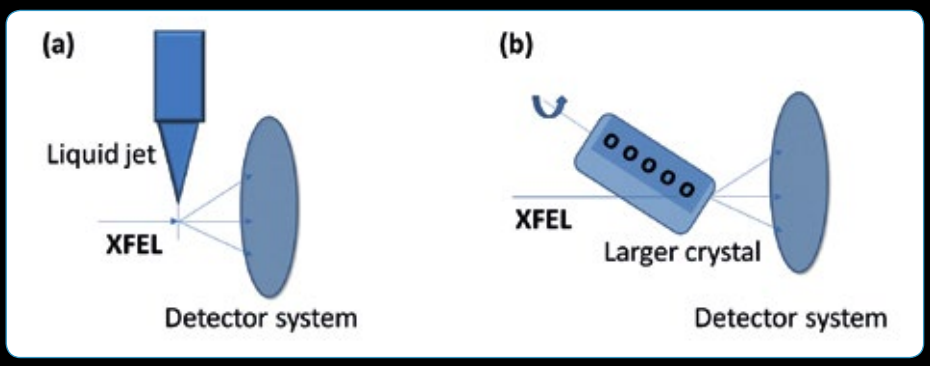


Fig. 2: Sample delivery: (a) in a liquid / gel jet, (b) as a static target

SCREENING
TRENDS IN DRUG DISCOVERY

25th Company Anniversary

In 2015 BMG Labtech celebrates its 25th company anniversary. Since the establishment in 1990, the company has become a specialist in microplate reading due to continuous development of innovative and unique technologies. Starting as an engineering office in Offenburg, Germany the Company has now subsidiaries in Australia, France, Japan, UK, and the USA.

www.bmglabtech.com

HAE Therapy

Cevec Pharmaceuticals, the developer and licensor of the CAP Technology, announced a major milestone regarding its recombinant human CAP cell derived C1 Esterase Inhibitor (C1Inh). The inhibitor material showed in a pivotal pharmacokinetic rat study a serum halflife matching one of the two currently available, plasma derived treatments, Berinert. All other known versions of recombinant C1 Inh, including e.g. Ruconest from transgenic rabbits, displayed so far a significantly shorter halflife than plasma derived C1 Inh. To achieve this protein quality of the recombinant C1Inh, new glycosylation optimized CAP-GO cells were developed. This result, in combination with previously shown specific activities and commercially attractive production yields using CAP-GO cells, paves now the way to develop a safer and more economic therapy for acute and prophylactic HAE indications.

www.cevec.com

Collaboration Announced

Artes Biotechnology, specialized in recombinant protein production, process and vaccine development from microbial expression systems announced a collaboration with the pharmaceutical company Boehringer Ingelheim Animal Health.

Artes' expression system *Hansenula polymorpha* is a technology for affordable mass vaccination and recommended by the World Health Organization (WHO) for these purposes. In combination with the Metavax platform, this offers a new approach to low-cost mass production of safe and effective vaccines required in the veterinary field.

www.artes-biotechnology.com
www.boehringer-ingelheim.com

AIMBE Fellow

Professor Jackie Y. Ying, Executive Director of the Institute of Bioengineering and Nanotechnology (IBN) of A*Star has been elected into the American Institute for Medical and Biological Engineering (AIMBE)'s College of Fellows. AIMBE Fellows are elites in the fields of medical and biological engineering. Prof Ying was nominated by her peers for her outstanding contributions to research and development of nanomaterials and nanosystems for biomedical application.

www.a-star.edu.sg
www.ibn.a-star.edu.sg

Paul Wallace as Chief Business Officer

Mission Therapeutics, a company focused on the discovery and development of modulators of the deubiquitylating (DUB) enzyme family for the treatment of cancer and other diseases, announces the appointment of Paul Wallace PhD as Chief Business Officer. Dr Wallace joins to formulate future business strategy at a time when the Company's pipeline has matured to the point where strategic options such as development partnerships are ready to be explored. Having worked in business development roles within drug discovery companies for nearly 20 years, Dr Wallace was most recently Executive Vice President and Head of Business Development at Medivir AB, where he was also a member of the Executive Management Team responsible for strategic and operational leadership, R&D portfolio management and investment planning. Prior to his roles in business development he had a career in pharmaceutical research and undertook his PhD and post-doctoral studies at the University of Cambridge.

www.missiontherapeutics.com

Call for Entries

Eppendorf and the journal Science are now accepting applications for the 2015 Eppendorf & Science Prize for Neurobiology. This annual international research prize of US$25,000 is awarded to young scientists for their outstanding contributions to neurobiology research based on methods of molecular and cell biology. Researchers who are 35 years of age or younger are invited to apply by June 15, 2015. The winner and finalists are selected by a committee of independent scientists, chaired by Science's Senior Editor, Dr. Peter Stern.

The 2014 prize was won by the US scientist Eiman Azim, Ph.D., Postdoctoral Research Fellow at Columbia University in New York. Eiman Azim's work offers fundamental new insights into the neural mechanisms that enable skilled limb movements to be both smooth and precise. His research has provided direct support for long-standing theories about the roles of internal feedback pathways within the central nervous system and external feedback from the muscles in regulating fine motor control.

www.eppendorf.com/prize

Initiative Announced

Peak Scientific announced a new initiative by which it will make a donation to a leading international charity that specializes in delivering emergency medical aid to areas of the world affected by conflict, epidemics, disasters or exclusion from health care.

When a customer purchases a Peak Scientific laboratory gas generator and registers to activate their free 12 month warranty, the company will donate $5 to the charity. Based on the substantial number of Peak generators sold around the world in 2014, this potentially could result in a substantial annual donation at the end of this year.

Warranty registration cards are included with all new instruments by the company and customers simply complete and return this or activate their warranty online. The warranty charity donation will be running throughout 2015 and at the end of the year the total amount of registrations received will be collated and a lump sum donation made to our chosen charity.

http://uk.peakscientific.com

Creating Biointerfaces

Bioactive Surfaces Enabled by Plasma Technology

Bioactive surfaces intend to cause a controlled effect at biointerfaces. For this purpose, well-defined properties of the material's surface are required. Plasma technology is well-suited to modify surface properties at the nanoscale, mainly by deposition of ultrathin coatings. Furthermore, a high level of understanding of biological processes at surfaces is essential. Some potential applications of bioactive surfaces are discussed for tissue engineering and antimicrobial effects. Plasma polymer films enable the functionalization of surfaces and immobilization of bioactive compounds as well as the controlled diffusion of drugs.

Introduction

The aim of bioactive surfaces is to induce a certain, intended response when exposed to bioorganisms. Examples are the support of cell growth, the inhibition of antimicrobial growth as well as the goal to suppress any attachment of bioorganisms, i.e. non-fouling surfaces. While there is increasing success in the first two fields, which are thus discussed in more detail, the latter goal remains challenging, since merely a delay in protein adsorption can be achieved up to date using, e.g., hydrogel-like coatings which hide surfaces by adsorption of abundant water molecules.

Nevertheless, the covalent immobilization of bioactive compounds onto material surfaces (often polymers) enabled considerable progress over past decades in diverse industries as biomedical, bioprocessing, microelectronics, food packaging, and textiles. Many novel life science applications are related to these developments from which, however, also challenges for the materials design occur requiring accurate processing, since precisely tailored surfaces and biointerfaces are mandatory. Plasma technology is thus investigated to functionalize surfaces for the attachment of bioactive compounds and living cells as well as to control the drug release by diffusion barrier layers. Therefore, mainly the deposition of thin films is considered, since film properties can be adjusted over a broad parameter range.

Plasma Technology

The plasma (as used in materials science) consists of a reactive gas containing ions, electrons, reactive species, and radiation. By means of electric or electromagnetic fields at high frequencies the plasma is generated at low temperatures (so-called 'cold' plasma) enabling the modification of temperature-sensitive surfaces (Fig. 1). Only a small part of the plasma is made of highly energetic particles causing ablation (etching or sputtering) or densification processes. Thin films can be deposited by the activation of monomers in the gas phase yielding plasma polymerization as well as by sputtering, i.e. the removal of atoms from a target material. Sputtering is mainly used to deposit metal (or metal oxide) films, since high sputtering yields can be achieved at low pressure. For plasma polymerization, different starting monomers (such as hydrocarbons and siloxanes) are used which can also be mixed with inert and reactive gases. Depending on the densification (cross-linking) during film growth highly functional surfaces or dense, hard coatings can be obtained. Plasma polymerization can both be performed at low pressure and atmospheric pressure.

Cell Growth at Surfaces

Cells or cell populations react to a broad spectrum of chemo-, mechano- physico-, and topological signals at a bio-material interface. As scaffold material for cell growth, therefore, a substrate is preferred that mimics the properties of tissue

Fig. 1: Plasma reactor used for plasma polymer deposition and co-sputtering from a metal target. The plane-parallel electrode set-up enables homogeneous plasma conditions. The gas flow is from the top to the bottom (pumping) side, while the samples are placed on the lower electrode.

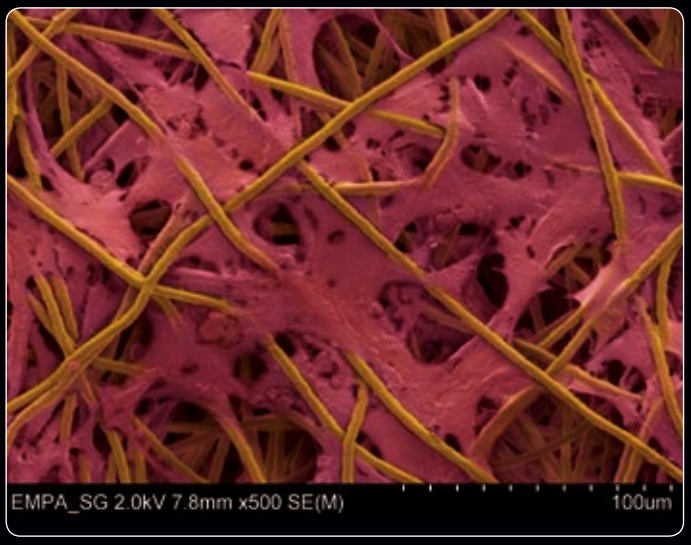


Fig. 2: Cell proliferation (mouse skeletal myoblasts) on a biodegradable, electro-spun scaffold. To enhance cell growth the substrate was coated with a nm-thick oxygen-functional plasma polymer.

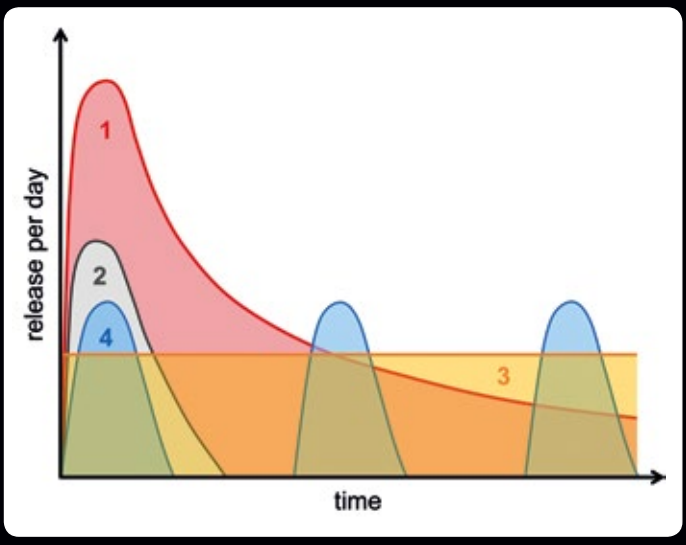


Fig. 3: Characteristics of different Ag release kinetics: 1) initial burst release causing local cytotoxic effects, 2) adjusted Ag release for short-term antibacterial effects (Ag is depleted afterwards), 3) steady state release using gradient layers, and 4) repeated Ag ion release mediated by degradable layers in a multilayer set-up.

(i.e. tissue engineering). Electrospun, fibrous polymer substrates are of particular interest due to their porous structure, use of biocompatible and/or biodegradable material, and low elastic modulus (comparable to tissue). Polymer surfaces, however, require a further functionalization step in order to obtain a bioactive surface. A simple plasma activation step (e.g. using an oxygen-containing plasma) might thus be used to obtain surface polar groups which, however, readily undergo reorientation processes. Deposition of thin films can avoid such aging effects when they are partly cross-linked (i.e. stabilized) yet still comprising a sufficient functional group density. For the coating of soft, tissue-like substrates the film thickness is limited to a few nanometers, since it has been shown that even plasma coatings exceeding ~10 nm substantially affect the mechanical properties (stiffness) of the substrate due to higher cross-linking. Therefore, ultrathin films are required that, in addition, do not show leaching of oligomeric compounds. Both, oxygen- and nitrogen-functional plasma polymer films have been extensively examined which contain hydroxyl, carbonyl, carboxyl and/or amine groups. While both coatings support cell growth at surfaces, oxygen-functional plasma polymers were found to have a higher stability and better penetration into a 3D-structured substrate. Cells can thus grow and proliferate at such modified surfaces (Fig. 2), which is increasingly used in tissue engineering.

Furthermore, both types of plasma polymers can also be used to covalently bind bioactive compounds (such as bio-linkers, growth factors etc.) at a surface.

Antimicrobial Surfaces

To obtain antimicrobial surfaces, mainly two approaches can be distinguished. While the immobilization of antibacterial molecules (such as quaternary ammonium compounds, polyphenols etc.) results in the inhibition of bacteria growth directly at the surface, the release of antibacterial agents also shows an effect on the surrounding media. The first approach is interesting for medical devices, displays, food packaging, and textiles. Again functional plasma polymer layers might be used for the immobilization of the bioactive compounds. The second approach based on drug release is mainly considered for wound care, catheters, sanitation, and implants. Here, mainly silver is used as the antibacterial agent due to its efficacy against a broad spectrum of bacteria and fungi. Furthermore, compared to copper or zinc oxide, silver (Ag) shows the lowest risk for human beings and the environment. The antibacterial effect is related to the release of Ag ions in aqueous media which interact with the metabolism, the respiration and replication system of microorganism. Nevertheless, high concentrations of Ag ions can also cause cytotoxic effects, i.e. cell populations become affected. Ag-rich surfaces typically yield an initial burst release of Ag ions which results in locally cytotoxic conditions (Fig. 3). To avoid such conditions but still enable antibacterial efficacy, the Ag ion release has to be adjusted. Using plasma polymerization, diffusion barriers can be deposited either on Ag-rich surfaces or during co-sputtering from a silver target. Gradients in the Ag content, i.e. more Ag in depth and less towards the surface, enable a steady Ag ion release. Some applications, e.g. implant surfaces, require a short-term Ag ion release or a recurrence of released silver over longer timeframes (see Fig. 3). Adjusted, Ag-poor coatings or multilayers of Ag-containing layers with degradable plasma polymers can fulfill the latter purpose.

While there is substantial progress in the field of antibacterial surfaces by fine tuning of the material's properties, different strains of the same bacteria were found to give a different response for the same antibacterial test conditions. Antibacterial tests should thus be repeated with different strains in order to enhance the reliability of the intended efficacy of a bioactive surface. Moreover, further understanding of the involved biological processes is required.

Outlook

Plasma technology has been shown to support cell growth, to immobilize bioactive compounds as well as to control the efficacy of antibacterial surfaces by avoiding cytotoxic effects. Beside the discussed contributions in tissue engineering and for antibacterial surfaces, bioactive surfaces are of growing interest for diverse fields such as biochips, biosensors, drug delivery, bioseparation, cell engineering, and stem-cell differentiation.

In order to obtain reliable materials and processes yielding well-defined bioactive surfaces, specialists from both fields of materials science and biology should further strengthen their collaborations. On one side, the performance of modified surfaces in a biological environment is under investigation, where plasma technology becomes an increasingly important tool, while on the other side, biologists steadily improve their know-how about important processes at bio-interfaces. Further progress in life science applications can thus be expected.

Contact
Dirk Hegemann
Empa, Swiss Federal Laboratories for Materials Science and Technology
St.Gallen, Schweiz
dirk.hegemann@empa.ch

United Kingdom Joins European XFEL

The United Kingdom will become the 12th member state of European XFEL, an international research facility that is currently under construction in the Hamburg area and will start user operation in 2017. The UK Minister for Universities and Science Greg Clark and the Science and Technology Facilities Council (STFC) announced that the United Kingdom will invest up to 30 M£ (about 38 M€) to become a full member. The European XFEL will produce extremely bright X-ray flashes that will allow scientists to investigate nanometre-scale structures, fast processes, and extreme states; take three-dimensional pictures of viruses and proteins; and film chemical reactions. The UK will become the 12th member of the European XFEL project, joining Denmark, France, Germany, Hungary, Italy, Poland, Russia, Slovakia, Spain, Sweden, and Switzerland. Overall construction costs are expected to be around £1.2 billion (in 2005 prices).

www.stfc.ac.uk

International Year of Light and Light-Based Technologies

The International Year of Light is a global initiative which will highlight to the citizens of the world the importance of light and optical technologies in their lives, for their futures, and for the development of society. It is an unique opportunity to inspire, educate, and connect on a global scale. On 20 December 2013, The United Nations (UN) General Assembly 68th Session proclaimed 2015 as the International Year of Light and Light-based Technologies (IYL 2015).

The United Nations has recognized the importance of raising global awareness about how light-based technologies promote sustainable development and provide solutions to global challenges in energy, education, agriculture and health. Light plays a vital role in our daily lives and is an imperative cross-cutting discipline of science in the 21st century. It has revolutionized medicine, opened up international communication via the Internet, and continues to be central to linking cultural, economic and political aspects of the global society.

www.light2015.org

Advancing Forensic Testing

Sciex announced a research collaboration with Labor Berlin, the largest clinical laboratory in Germany, for the development of a hybrid Quadrupole Time-of-Flight (TOF) MS/MS reference library of relevant forensic chemical compounds. The library will cover thousands of chemical substances, allowing users of Sciex TripleTOF 6600 mass spectrometers for forensic testing to more effectively and efficiently develop and validate new analytical methods for forensic compound screening. The reference library generated by this collaboration will ultimately allow forensic scientists to identify and analyse unknown substances or toxins, including pharmacological agents or forensic drugs in samples more easily, accurately and with more confidence.

The generation of the hybrid Quadrupole TOF library of chemical substances used in conjunction with mass spectrometry techniques is particularly important for applications in forensic toxicology. Forensic samples may contain unknown substances that a person may have ingested up to several weeks prior to the sample being taken. Therefore, forensic testing requires a precise, sensitive and robust approach to sample analysis.

Labor Berlin will provide the pharmacological input to the collaboration and Sciex will assemble the hybrid Quadrupole TOF library using its expertise and robust and reliable instrumentation.

http://sciex.com

www.laborberlin.com

New Management

At the beginning of March a new company management at Carl Zeiss Microscopy was announced. Dr Markus Weber and Justus Felix Wehmer have taken over in dual leadership, replacing Dr. Ulrich Simon who was the first leader of the Microscopy division of Carl Zeiss founded in May 2012, when the individual business units of electron and optical microscopy were combined. The physicist Ulrich Simon has now been appointed CEO of the Research & Technology unit of the corporate group. Thereby switching positions with the physicist Markus Weber, who will be responsible for the development of products and technology for the Microscopy group. Additionally the economist Justus Felix Wehmer, former head of commercial management in the Zeiss SMT division, will focus on finance, sales and services in the Microscopy unit.

www.zeiss.de/microscopy

Controlling Temperature With Ease

Compact Temperature Control Units

Typical laboratory applications include sample preparation, cooling water supply, reaction control, thermal separation processes, analyses and material inspection require absolutely precise temperature control. Therefore, heating and cooling devices are a part of the basic equipment in most laboratories. The Huber product range offers heating and cooling thermostats and circulating coolers with features and functionality tailored to the needs of a laboratory.

Robust, Easy to Use and Cost-effective

The Huber temperature control program offers over 200 standard models for applications in research, technical centers and production. Of particular interest to laboratory users are the space-saving Minichillers and Ministats as well as the Petite Fleur models and the cost-effective MPC thermostats.

Circulating Cooler

The Minichillers are compact circulating coolers with a capacity of up to 300 watts and working temperatures from -20 °C to +40 °C. The devices are rugged and inexpensive and have a footprint of only 225 x 360 mm. On request, the cooler are available with RS232 interface and built-in heater (1 kW) and an extended temperature range up to +100 °C. Minichillers are water or air cooled and all models operate environmentally friendly with natural refrigerant. Typical laboratory applications are rotary evaporators, distillation equipment, microscopes and analytical apparatus and measurement devices.

Refrigeration Circulators

The Ministats are the smallest cooling circulators available on the market and are suitable for even more applications. Its dimensions enable the device to operate in very limited lab-space, for example in a laboratory extractor hood. Ministats are suitable for the thermoregulation of photometers, refractometers, viscometers, reaction vessels and mini-plant systems. Although the focus is on external applications, the bath opening is sufficiently large to temper smaller objects in the circulator bath.

The Ministat series consists of three models, each of which is available as air or water cooled unit. Depending on the model, working temperature ranges from -45 °C to +200 °C and a cooling capacity of up to 600 watts is achieved. The maximum permissible ambient temperature is +40 °C. An optimum circulation is provided by an adjustable pressure-suction pump. Optionally, the maximum pressure can be regulated as well - thus effectively protecting sensitive glass reactors from breakage. Ministats are equipped with the modern Pilot ONE controller as a standard. The controller has a 5.7 inch TFT colour display with a comfortable, smartphone-like user interface in 11 languages. Included are two USB ports as well as LAN and RS232 ports. Another plus point is the electronic upgrade function for the activation of additional functions such as programmer, cascade control, ramp function, user menus, calendar start etc. Analogue NAMUR connections can be upgraded via the optional ComG@te module, thereby also providing integration with process control systems.

The new Grande Fleur for temperature control of research reactors with high cooling and heating rates.

Fig. 1: Ministars are the smallest refrigeration circulators in the world.

Fig. 2: Pilot ONE controller with colour display, graphical temperature display and USB and LAN ports for remote control and data recording.

Fig. 3: Simple touch-screen operation of Pilot ONE models: memorable icons and a customizable Favourites menu make the operation intuitive and self-explanatory.

Dynamic Temperature Control Systems

Another highlight in the product portfolio are the „little Tangos". The small Tangos are the entry models into the world of Unistats: Due to their compact dimensions and unique thermodynamics, Petite Fleur and Fleur Grande are ideally suited for the high-precision thermoregulation of research reactors.

Grande Fleur

Larger than a Petite Fleur but smaller than a Unistat Tango, the Grande Fleur expands the product range and offers even more performance at an affordable price. Both models, Petite and Grande Fleur, offer all of the performance characteristics and features of the Unistat series such as USB, Ethernet and RS232 interfaces, the touch screen controller Pilot ONE, process data recording via USB as well as natural refrigerants and thermodynamics, which are second to none. The working temperature range is from -40 °C to +200 °C. A cooling capacity of 600 watts according to DIN 12876 is available at full pumping capacity. If the pump speed is reduced, the cooling capacity is further increased by 50 watts. An advantage, especially for frequently changing applications in the laboratory, is the water separator system that removes residual water from the temperature control circuit in hoses and reactors. With flow rates of up to 38 l/min, efficient heat transfer is provided by the continuously variable circulation pump. The gentle start of the pressure control VPC protects glass reactors from damage and automatically compensates changes in the viscosity of the temperature control medium. The system therefore ensures minimum pressure with maximum circulation and thereby optimizes the heat transfer to the application. Both Fleur models are equipped with the previously described Pilot ONE controller. The units for the thermoregulation of externally closed temperature control circuits or externally open applications are available in two versions.

Affordable Bath Thermostats

The offers are complemented with classic bath or circulation thermostats. The models are suited for external applications or for direct thermoregulation in the open bath. Two equipment lines are available for heating and cooling thermostats. In addition to the models with Pilot ONE controller, the inexpensive MPC model series may be interesting for many routine laboratory tasks. The thermostats are available with stainless steel or transparent polycarbonate baths for working temperatures from -30 °C to +200 °C. If a bath vessel already exists, immersion thermostats with screw clamps or bridge thermostats with an extendable telescopic bridge are available. Accessories such as test tube racks, shelves and batch covers make many everyday temperature control tasks easier.

Fig. 4: Robust Minichiller for cooling applications in the lab like rotary evaporators, vacuum pumps, distillation equipment, etc.

Free Software

A useful extension for the visualization and documentation of process-relevant data in connection with Huber temperature control units is the free SpyLight software. The software runs under Windows and is suitable for data recording or for remotely controlling the temperature control unit via a PC, laptop or tablet. The communication can use RS-232, RS485, USB and TCP/IP. The recorded data are plotted against time with the axes of the diagram being freely scalable. The zoom function also simplifies the graphical analysis of individual time periods. SpyLight features ease of installation, low resource consumption and ease of operation. The free version is not time limited and is compatible with all Huber temperature control units using the Pilot ONE and MPC controller. The software is available as a free download in German and English on www.huber-online.com.

Contact

Peter Huber Kältemaschinenbau GmbH
Offenburg
Tel.: 0781/ 9603-0
Fax: 0781/57211
info@huber-online.com
www.huber-online.com

PCR Optimization

Finding the Optimal Primer Annealing Temperature

The base composition and length of PCR primers generally determine the annealing temperature of primer pairs. New primer pairs are designed to anneal at a specific temperature by calculating the primer melting temperature (T_m). The T_m is the temperature, at which one-half of a particular DNA duplex will dissociate and become single strand DNA. The stability of a primer-template DNA duplex can be measured by its T_m and new primers are usually supplied with an additional data sheet providing information on the calculated theoretical melting temperatures (T_m).

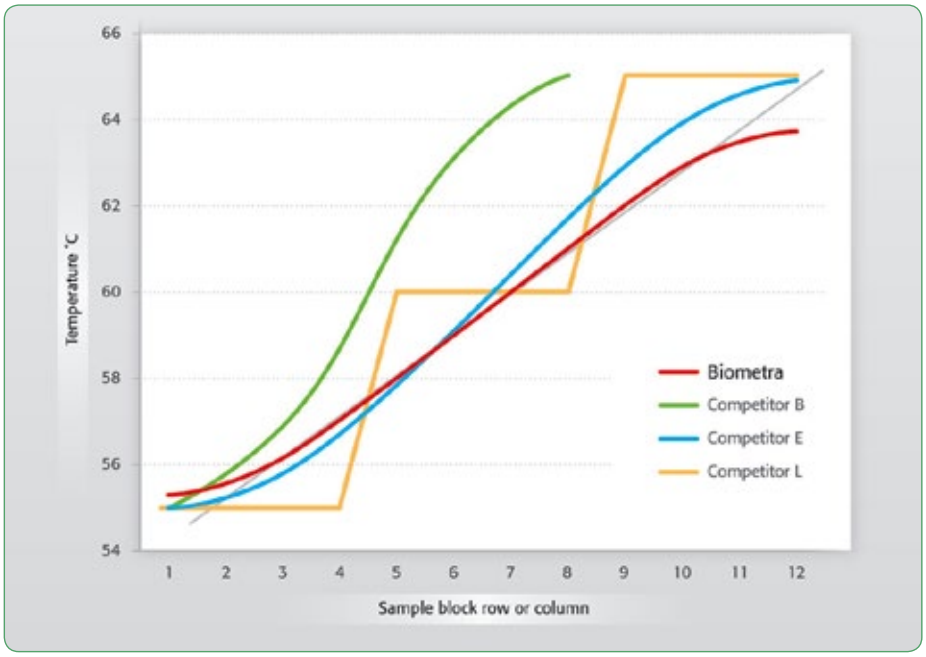


Fig. 1: Diagram showing the sample block temperatures with gradient programmed from 55 °C to 65 °C using a temperature difference (increment) of 1°C per lane. For Biometra thermal cyclers the temperature difference from lane to lane is exactly the same from lane 3 to lane 10 as indicated by the dotted grey line. Competitor E also applies the gradient along the long side of the sample block but the temperature difference from lane to lane is different, leading to a sigmoid shape of the temperature curve. Also for Competitor B the temperature difference from lane to lane is not the same. Additionally the gradient is applied along the short side of the sample block leading to a lower number of available different temperatures. For Competitor L the temperature is exactly the same for a single temperature zone but the number of different useable temperatures is greatly reduced.

There are a number of different algorithms to calculate the estimated T_m of a primer that may give widely varying results. The simplest methods are the basic T_m calculations according to Marmur and Doty (1) or Wallace *et al.* (2). For these methods neither the salt concentration nor the position of the nucleotides in the primer sequence are considered and thus the algorithms are not meant to be used for sequences longer than 14 nucleotides or give questionable results for longer primers. By salt adjusted T_m calculations (3) at least the salt concentration is considered. The best methods to calculate primer T_m values are base-stacking T_m calculations (4, 5, 6). The base-stacking formulas use the nearest neighbor algorithm and calculate the primer T_m-value based on the nucleotides position and salt concentration.

Temperature Constancy

Primers are usually supplied inclusive information on the calculated theoretical T_m. However, the different T_m-calculation methods give widely varying results and the precise optimum annealing temperature (T_a) has to be determined experimentally. In general the T_a is set 5°C below the mean T_m-value of the primer pair. From expe-

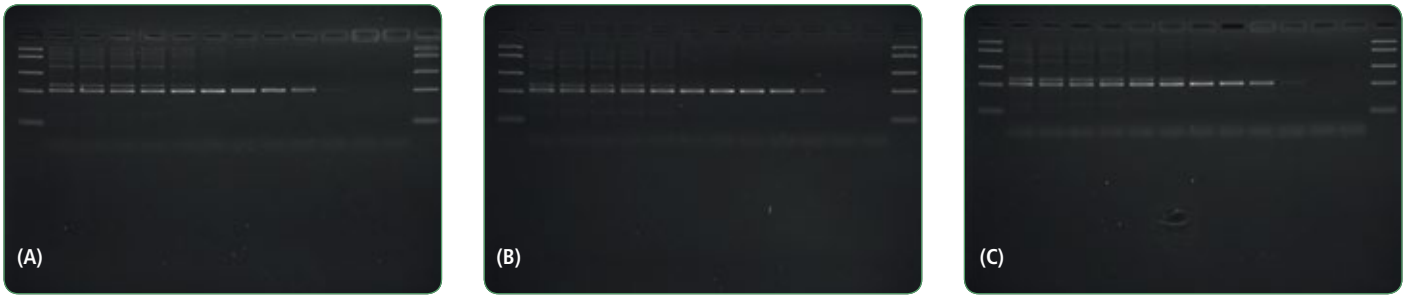


Fig. 2: Gradient PCR using different primer concentrations. A human b-globin fragment was amplified using primers RS41 and RS42 at concentrations of1:1 (A), 2:1 (B) and 1:2 (C).

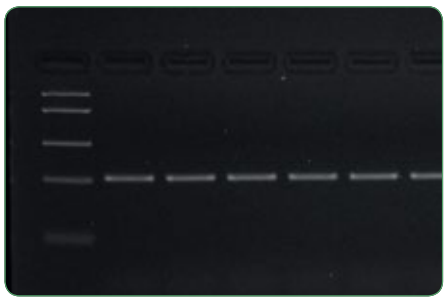


Fig. 3: Results of gradient PCR (A-B) were transferred to a standard PCR reaction at 60°C using a primer concentration of 2:1.

Tab 1.: Temperature and time protocol for gradient PCR

Step	Cycles	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	96 °C	2 min	max
2	30	Denaturation	96 °C	10 sec	max
3		Annealing	56°C ±3.0°C	10 sec	max
4		Elongation*	72 °C	20 sec	max
5	1	Final Elongation	72°C	1 min	max

riences using the equation T_a = mean T_m - 5°C most often leads to formation of a PCR product in the first PCR experiment. However, the calculated mean T_m-value and the optimal annealing temperature can differ by more or less than 5°C and using a too low annealing temperature can lead to the formation of non-specific products whereas if the annealing temperature is set too high the PCR yield may be reduced or no PCR products are formed.

By using the gradient function of a PCR thermal cycler the optimal primer T_a can be determined which is then used as integer temperature value (T_a-value) in routine PCR protocols. Traditionally gradients are programmed by setting temperature values for the left and right side of the sample block (e.g. 55°C-65°C). Due to technical reasons the temperature gradient of most PCR instruments has a sigmoid shape what means that the temperature difference from lane to lane differs across the sample block (Fig. 1). The traditional way of gradient programming and the unequal temperature differences between the lanes lead to disadvantages when trying to find the optimal primer annealing temperature (T_a).

Some thermal cycler models use thermal zones. Here it is possible to set a defined temperature for a specific zone. However, for each temperature zone the temperature value has to be set individually and the overall number of temperatures is reduced compared to gradient enabled thermal cyclers.

The mentioned disadvantages are overcome by the linear gradient tool (Biometra). This tool allows entering the calculated T_a-value and offers to set integer temperature values with constant difference (increment) from lane to lane. For example, a calculated primer annealing temperature (T_a) of 60.0°C can be programmed for lane 6 of the 96 well sample block and the temperature difference between the sample block lanes set to ±1.0°C. For the 96 well sample block in total 12 different lanes (temperatures) are available.

Finding the Optimal Primer Annealing Temperature (T_a)

As mentioned above usually the primer annealing temperature is calculated from the mean primer melting temperature using the following equation: T_a = T_m-5°C. In practice the optimal primer annealing temperature cannot be calculated using a formula but has to be determined experimentally. As a starting point the calculated primer T_a and a temperature increment of ±1.0°C should be used to program the gradient. If necessary the increment can be reduced for fine-tuning in later experiments. In the gradient step different primer concentration combinations can be used to find the best annealing temperature and primer mix simultaneously.

In the following example a 210 bp b-globin fragment has been amplified from human cDNA. To demonstrate the effect of the annealing temperature on the PCR results the gradient has been programmed using an annealing temperature of 56°C (lane 6) and a temperature increment of ±3.0°C. Additionally the forward and reverse primer has been in used at concentrations of 1:1 (Fig. 2A), 2:1 (Fig. 2B) and 1:2 (Fig. 2C). The best results were obtained using a primer concentration of 2:1 and an annealing temperature of approximately 60°C.

The results were transferred to a standard PCR program to demonstrate the specificity and robustness of the results. When using a primer concentration of 2:1 and an annealing temperature of 60°C the PCR yields a single product in all 12 lanes of the sample block (Fig. 3).

Tab 2: Temperature and time protocol for standard PCR

Step	Cycles	Profile	Temperature	Holding time	Ramp rate
1	1	Initial denaturation	96 °C	2 min	max
2	30	Denaturation	96 °C	10 sec	max
3		Annealing	60°C	10 sec	max
4		Elongation*	72 °C	20 sec	max
5	1	Final Elongation	72°C	1 min	max

Methods and Materials

For PCR the Analytik Jena innuTaq Hot-A DNA-Polymerase has been used according to the user manual. As template Promega human DNA has been used at a final concentration of 0.5 ng/ul. The human β-globin fragment was amplified using forward primer RS41 and reverse primer RS42 at varying concentrations of 1:1, 2:1 and 1:2 (0.5 µM to 1.0 µM). For gradient PCR (A-C) the Biometra TAdvanced thermal cycler the PCR program shown in Table 1 was used, for standard PCR see PCR protocol in tab. 2.

Conclusion

The linear gradient tool is a useful feature to find the optimal primer annealing temperature (T_a) in a single PCR experiment. It allows to enter the calculated primer annealing temperature and to define the temperature increment. One can obtain gradients with even temperature values from lane to lane and to transfer the gradient step results into a standard PCR protocol. Thus it saves the user time and effort when optimizing new primer pairs.

References

[1] Marmur J and Doty P J Mol Biol 5:109-118 (1962)
[2] Wallace *et al.*: Nucleic Acid Res. 6: 3543 (1979)
[3] Rychlik W. and Rhoads, R.E. Nucl. Acids Res. 17, 8543 (1989)
[4] Breslauer *et al.:* Proc. Natl. Acad. Sci. USA 83: 3746 (1986)
[5] SantaLucia J. Proc. Nat. Acad. Sci. USA 95, 1460 (1998)
[6] von Ahsen N. *et al.:* Clin. Chem. 45, 2094 (1999)

Authors
Holger Densow, Martina Pick, Analytik Jena AG, Business Unit Life Science, Biometra Product Line

Contact
Analytik Jena AG
lifescience@analytik-jena.de
www.bio.analytik-jena.de

Water Contaminants Analysis

Analysis of Drinking, Surface or Waste Water

In the analysis of contaminants in water, both volatile and semivolatile compounds are detected as well as a variety of compounds such as pesticides, PAHs, PCBs, PBDEs and others. A GCMS/MS setup provides a highly sensitive and very fast solution.

In the analysis of contaminants in drinking, surface or waste water, the analytes to be checked can be subdivided into volatile and semivolatile compounds (VOC, SVOC) and other regulated substances. For VOCs and SVOCs thermal extraction techniques such as headspace (HS) or HS-solid phase microextraction (HS-SPME) are applied. Depending on the relevant regulation, compounds like pesticides, polyaromatic hydrocarbons (PAHs), polycyclic biphenyls (PCBs), phenols, anilines and polybrominated diphenyl ethers (PBDEs) are prepared using liquid-liquid extraction with subsequent SPE and concentration steps before liquid injection into the GCMS/MS. The last concentration step can be done in the GC injector (Programmed Temperature Vaporizer (PTV)) and is referred to as large volume injection (LVI).

It is possible to run both types of analysis on the same instrument without mounting or switching columns. The following paragraphs show data obtained with Headspace injection (VOC) as well as results of a series of compounds in water which were injected into a PTV as liquid after extraction. The injection volume was 50 µl.

Experimental

For the two types of target compounds indicated above, different GC columns had to be used. This usually needs an additional instrument or column switching technologies. The fast analytical method described here applies an RTX-624 (Restek), 20 m, 0.18 mm, 1.0 µm column mounted for the VOC analysis whereas an RTX-5 MS (Restek) 30 m, 0.25 mm, 0.25 µm column was used for compounds injected as liquid extracts.

With a twin line kit (Shimadzu) and without additional tubing, the two columns were mounted simultaneously on the MS as part of the GCMS-TQ8040 (Shimadzu) triple quadrupole instrument.

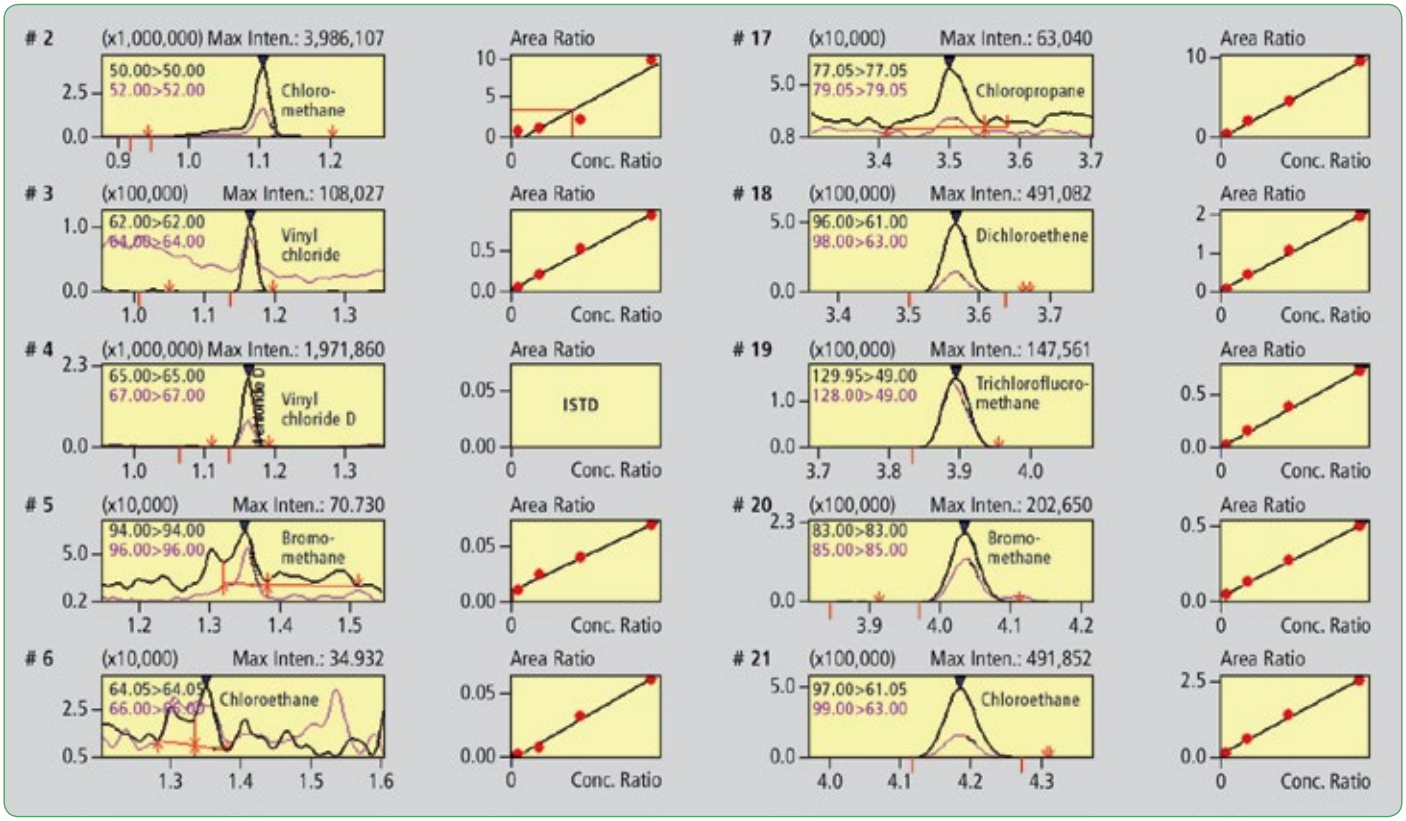


Fig. 1: Fast Data acquired with water spiked with a 50 ng/l VOC standard

Both columns have their own injector and end side by side at the same optimum position in the source. With the RTX-624, a split/splitless injector was used. The second column was mounted to a PTV (Optic-4; GL Sciences). Analytical runs of either type were done sequentially in one batch.

As autosampler, a xyz-robot (AOC-6000, Shimadzu) was applied with the option to change the syringe automatically from liquid to headspace injection modes. To achieve fast headspace analysis, a cold trap (cryofocus-4, GL Sciences) was placed below the injector to refocus the compounds on the column [1].The cold trap refocused at a temperature of -150 °C, and the compounds were released from the trap at a heating rate of 50 °C/sec up to 240°C.

20 ml vials were filled with 5 ml water to which 3 g of sodium chloride was added. Thermal extraction was executed at 60 °C incubation for 20 minutes. The injection volume was 1 ml in the headspace of the vials using a split ratio of 5:1. Whereas in reference [1] a single quadrupole instrument was used to analyze the VOC compounds, in this case a triple quadrupole instrument was utilized for all targets. For the nonvolatiles except PAHs, true MRM transitions were applied (Q3 set m/z different from Q1), the VOCs and PAHs (the latter partly) were recorded in the so called pseudo MRM mode [2] where Q1 set m/z = Q3 set m/z with small or zero collision energy.

The resolution of the GCMS was run in unit (Q1)-low (Q3). With this combination, sensitivity and selectivity was optimized. Dichloromethane was used as solvent of the liquid samples. Injection volume was 50 µl injected at 50 °C PTV temperature and a split ratio of 100:1 for 30 seconds. The split was then closed and the PTV was heated to 280 °C. As some pesticides degrade quite easily in the injector, a sintered glass liner was used as insert, and injection was done applying a needle with side hole [3].

Results

VOC analysis

Figure 1 shows selected VOC compounds recorded with a water sample spiked with 0.05 ppb concentration together with MRM transitions and calibration curves (internal standard). In total, 75 compounds were analyzed in one run. Total run time was less than 11 minutes.

The peak relating to vinyl chloride has a peak width at the base of 1.2 sec which indicates the effectivity of refocusing [1]. For that compound Q1 (m/z) = Q3 (m/z) with collision energy CE = 0. In the same figure, the peak of trichlorofluoromethane is presented where Q1 (m/z), Q3 (m/z) was set to 129.95, 49 Da at 21 eV collision energy (Ar). Table 1 summarizes the list of compounds with transitions and S/N ratios (RMS) (see http://bit.ly/VOCs_Tab1).

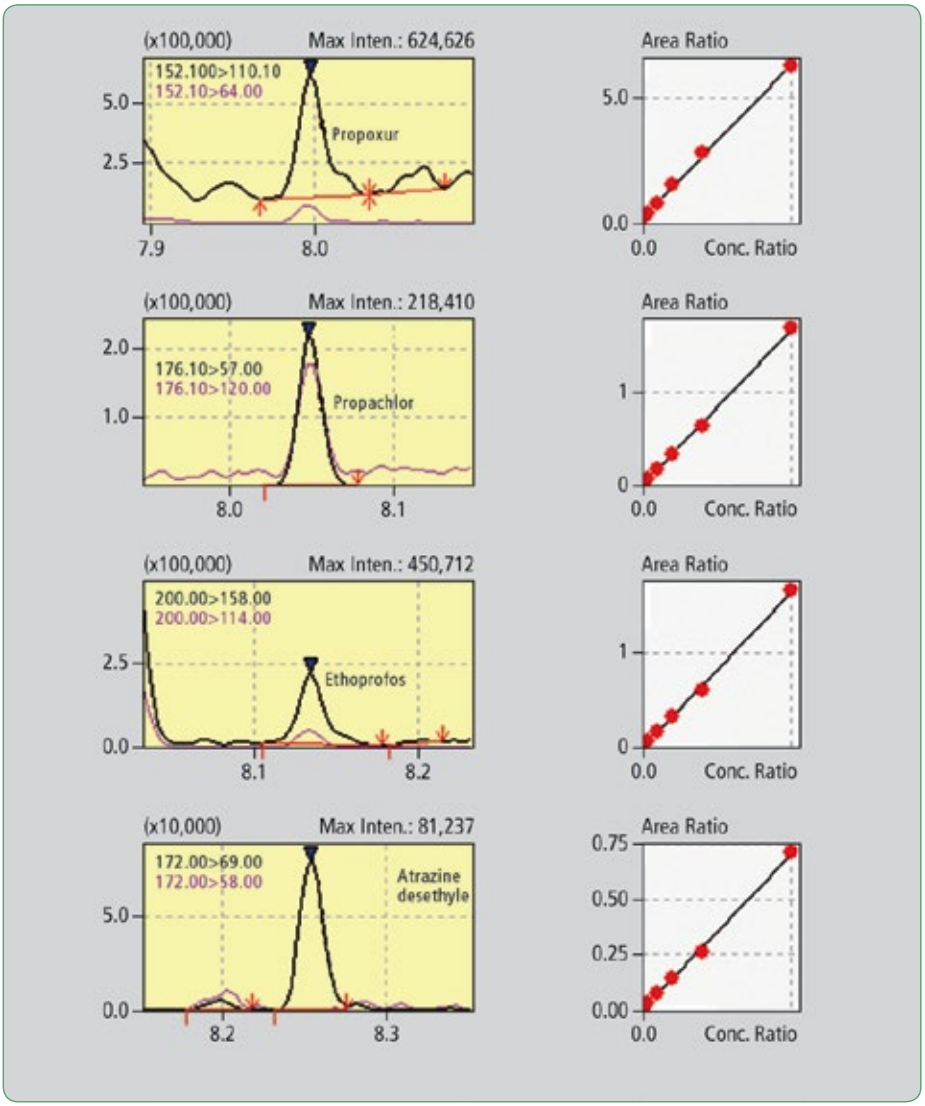


Fig. 2: Selected compounds of an extracted water spiked with 0.02 pg after liquid injection of 50 µl.

For the compounds measured with pseudo MRM, sensitivity is comparable with selected ion monitoring (SIM) which was recorded using the same instrument. For the other peaks, sensitivity in MRM mode is much higher than in SIM.

Liquid injection

Figure 2 shows Propoxur, Propachlor, Ethoprophos pesticides and atrazine-desethyl as examples recorded with a spiked water sample together with calibration curves (internal standard). In total, 51 target compounds were measured, with a run time of ~ 25 minutes. The amount of each compound injected was 0.02 pg as the data shows. The limit of detection from the peaks could be estimated as below 0.01 pg.

Conclusion

A water analyzing GCMS/MS setup was used to determine VOCs with headspace GCMS/MS and contaminants such as pesticides, PAHs, PCBs etc in one triple quadrupole GCMS by using a twin line kit. The limit of detection for the VOC compounds was well below 10 ng/l. For the target injected with liquid large volume of 50 µl, the detection limits were below 10 fg in the extract.

Literature

[1] Schröder S. *et al.:* LCGC, The column, Mar 2nd (2012)
[2] Schulte H. *et al.:* Poster International Symposium on capillary chromatography Riva, Italy (2014)
[3] Friedrichs K. *et al.:* LCGC The column, Mar 2nd (2009)

Author
Dr. Hans-Ulrich Baier
Product Specialist, GCMS, Shimadzu Europa GmbH

Contact
Shimadzu Europa GmbH
Duisburg, Germany
shimadzu@shimadzu.eu
www.shimadzu.eu

Determination of Hexavalent Chromium

Metrohm has developed ion chromatography determination methods for determining Cr(VI) in various concentration ranges (ng/l to mg/l) with inline sample preparation techniques for various matrices. Applications are the Determination of chromium(VI) in mineral and drinking water; Cr(VI) in toys – Determination by ion chromatography; and Cr(VI) in leather – Determination by ion chromatography and Inline Dialysis. Hexavalent chromium or Cr(VI) is classified as allergenic, carcinogenic and extremely toxic, and is subject to strict monitoring. Cr(VI) can occur in various concentrations in different areas, e.g. drinking water, toys and textiles.

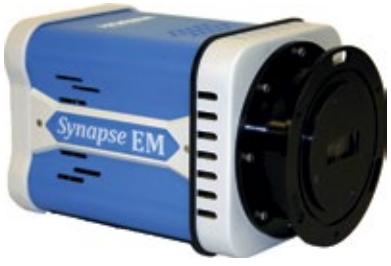
Metrohm
www.metrohm.com

Fitting Range Extended

Diba Industries adds the 10-32 cone-tip Click-N-Seal Ultra and the 6-40 Click-N-Seal Micro to their existing range of fittings. Both fittings utilize the Company's patented Click-N-Seal technology: the fitting is hand-tightened until it reaches the correct torque, at which point the cap clicks. This ensures leak-free connections without the risk of under- or over-tightening. The 10-32 cone-tip Click-N-Seal Ultra connects 1/16" OD semi-rigid tubing, such as PTFE, ETF, FEP and PEEK, in to 10-32 conical ports. The fitting is suitable for pressure ratings up to 2000psi (140 bar) and is constructed from PEEK for enhanced chemical resistance.

Diba Industries
www.dibaind.com

Deep-cooled Scientific Spectroscopy Camera



Horiba Scientific launched its Synapse EM, a deep cooled EMCCD Scientific camera for low light and ultra-fast spectroscopy experiments. It is available in front and back illuminated for optimal quantum efficiency. With a sensor format of 1600 x 200 or 1600 x 400 and a pixel size of 16 microns, it is suited for high spectral resolution measurements. The camera comes with a standard dual readout mode that allows the users to switch automatically between EMCCD mode for low light measurements and conventional CCD mode for standard spectroscopy. There are several sensor options including the Qextra technology. This technology is an anti-reflective multi-layer coating designed to improve quantum efficiency of back illuminated sensors over a broad spectral range, while suppressing undesirable etaloning effects.

Horiba Scientific
www.synapseem.com

Pipetting Assistant

The semi-automatic electronic pipette Cybi-Selma is equipped with 96 or 384 parallel working tips with a volume range from 500 nl to 1 ml. Based on Cybio's tip sealing technology, it offers error free and reproducible results. Automatic tip tightening avoids laborious adjustments and preloaded Cybi-Tip-trays enable rapid execution within seconds. An open design allows the processing of any 96- or 384-well microplates as well as the use of accessories like shakers. Features include Intuitive operation via multilingual touch screen (German, English, Chinese, Japanese, Russian); a comfortable selection of different pipetting parameters and saving of methods; processing of individual columns and dilution series, and the integration of accessories like shakers.

Analytik Jena
www.cybio-ag.com

Enhanced qPCR Performance

Bibby Scientific has announced that Techne, a UK manufacturer of laboratory benchtop equipment, has launched its qPCR system, the Prime Pro 48. It offers greater accuracy and higher quality data for a variety of applications. The system has 400 associated qPCR detection kits, which cover a wide range of application areas including clinical, veterinary, food, and biohazard testing. The economical 48-well PCR plate is simple and fast to set up. The smaller plate size and patented block technology deliver very high thermal uniformity, providing more consistent, higher-quality data. This means researchers can use duplicates rather than triplicates, which increases throughput, saves money and reduces waste of precious DNA template.

Bibby Scientific
www.bibby-scientific.com

Temperature Control

Huber's Unistat temperature control systems are tailored to applications in process engineering. They perform accurate temperature control for very small batches or for production volumes in a range of -125 °C to +425 °C. The system allows consistent scale-up in research, kilo-lab, miniplant, technology center and in production. More than 60 models and 200 variants with cooling powers of 0.7 to 130 kW are available. Furthermore, the temperature control systems may be combined with vapor or cooling brine circuits; this makes them suitable for production volumes beyond the 10 m^3 class. When requirements increase, the instruments can easily be adapted, while their mode of operation and general functionality remain the same. A wide range of setting options allows the user to adjust the temperature control and regulation behavior exactly to the individual application.

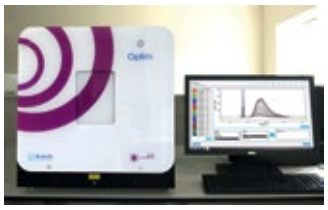
Peter Huber Kältemaschinenbau
www.huber-online.com

Grinding Tough and Fibrous Samples

Retsch has introduced a serrated blade knife for its Knife Mill Grindomix GM 200. Grinding and homogenizing of tough and fibrous food samples can put a man's patience truly to the test, the manufacturer says: It frequently happens that part of the sample escapes the knives or some material wraps itself around the knife blades. As a result, the sample is only insufficiently homogenized which may lead to flawed analysis results. Thanks to the serrated blades the new knife ensures complete homogenization of tough, gristly and fibrous samples such as, for example, meat or streaky bacon, thus greatly improving the sample preparation process.

Retsch GmbH
www.retsch.com

Analyzer for Membrane Protein Stability Tests



Avacta Analytical has extended the versatility of the Optim 2 protein stability and characterization instruments. A version featuring an additional 375 nm laser enhances the measurement of stability information for membrane proteins. The capability of the Optim 375 was recently evaluated in the Faculty of Biological Sciences at the University of Leeds. It can be used to effectively guide purification and biophysical characterization efforts, including crystallization, by tracking the exposure of cysteine residues within the protein interior as an indicator of protein unfolding, with the ability to simultaneously follow protein aggregation using the system's built-in static light scattering.

Avacta Analytical
www.avactaanalytical.com

High Brightness Deuterium Light Source Module

Hamamatsu Photonics introduce their high brightness deuterium (H2D2) light source, featuring a deuterium lamp that emits UV light at intensity six times higher than the Company's conventional L2D2 range of lamps. Even with this increased brightness the lifetime of the lamp is not comprised, with 1000 hours guaranteed. The stability remains at 0.05% peak-to-peak. The lamp only requires air-cooling when used in the dedicated housing. The manufacturer has also produced an optimized power supply for best possible use of the lamp. The lamp is available with either MgF2 or synthetic silica window for a choice of UV output.

Hamamatsu
www.hamamatsu.com

Spectrometers for Elemental Analysis

Spectro Analytical Instruments presents its Arcos high-resolution ICP-OES spectrometer, featuring the fast and convenient selection of axial plasma or radial plasma observation in a single instrument. Designed for use in industry, science, and academia, the spectrometer surpasses the performance limitations of conventional ICP-OES instruments —improving sensitivity, stability, and precision, while lowering operating costs with the introduction of innovative components, unique capabilities, and optimum flexibility. Features include axial or radial plasma observation, the ORCA Optical System (CCD optic system with Paschen-Runge mount). The system makes purge gases unnecessary and there is no external cooling system needed.

Spectro Analytical Instruments
www.spectro.com

Spectrofluorometer Combined with Cryostat

Edinburgh Instruments has combined its FS5 Spectrofluorometer with a liquid nitrogen cryostat to achieve a range of significant sample measurement capabilities with a single click. This enables users to measure the spectral properties of a sample at any temperature from 77 – 500 Kelvin, suited for phosphorescence or delayed fluorescence measurements where samples are sometimes frozen at liquid nitrogen temperatures in order to preserve the fragile triplet state. This enables sample measurement and analysis that is not otherwise possible. Automated three-dimensional measurements are possible – in order to produce temperature dependent maps of excitation, emission, synchronous scans, phosphorescence decays and fluorescence decays. Users can also measure samples of different states including solid crystals, thin films, powders and liquids using a variety of sample holders.

Edinburgh Instruments Ltd.
www.edinst.com

Industrial Weighing

Mettler Toledo has introduced a line of Industrial Basic Scales, a portfolio of rugged scales with features needed for accurate, reliable weighing for dry and wet environments. It comprises the following products: Basic portioning scales for fast, mobile food weighing; checkweighers for food and manufacturing applications; rugged floor scales and pallet-weighing solutions for logistics and warehousing applications, and reliable basic counting scales for accurate order picking, packaging and completeness checks. The scales come with stainless or mild-steel weighing platforms, easy-to-read terminals and secure overload protection.

Mettler Toledo AG
www.mt.com

Planetary Mill

The new Fritsch premium line offers a new dimension in high-tech milling. For the first time, rotational speeds and ultrafine grinding results down into the nano range are achieved.

With this planetary mill one can achieve rotation speeds of up to 1100 rpm and an acceleration of 95 times the force of gravity. The bowl and lid form a solid unit – the grinding bowls are closed gas-tight with one motion and with a second motion, they are safely locked in the mill. The grinding bowls position themselves and snap securely into place this means no additional tensioning and no incorrect operation. The grinding chamber opens and closes automatically and independently rotates the bowl mounts in a convenient position for handling.

Safety: The mill automatically detects the inserted grinding bowls via a special RFID chip, then optimizes the rotation speed and prevents impermissible grinding settings.

Fritsch GmbH
www.fritsch-milling.com

Tracking Immune Function

Qiagen announced the commercial launch of Quantiferon Monitor (QFM), a diagnostic for monitoring immune function. Primary applications include monitoring of immune function in solid organ transplant recipients. The product measures the cell-mediated immune response and can provide important information on the strength of the immune system in the immunosuppressed solid organ transplant population. The test thereby targets an important medical need of physicians who need to assess patients' risk for both organ rejection and infections in order to determine the right dosage of immunosuppressive drugs. Currently, best practice in assessing immune reactivity is to monitor levels of those drugs. However, today there are no standard drug regimens applied to all patients.

Qiagen
www.qiagen.com

Sensors for Flow Rate Measurement

Sartorius Stedim Biotech has entered into a global development cooperation agreement with the flow measurement company Emtec. This specialized firm provides non-invasive flow engineering solutions for blood vessels and flexible tubing. Such technology is used in the biopharmaceutical industry, among other sectors, in many process steps for accurate measurement of flow rates and mass balances. In particular, this technology is used in downstream processes, such as virus inactivation, as well as in ultrafiltration and diafiltration. As part of this cooperation, the Company's technology base will be extended by jointly developed single-use components, such as Flowtube. Sartorius will exclusively market these as applications and distribute them under the brand name of Biopat Flow.

Sartorius Stedim Biotech
www.sartorius.com

Analysis of Delicate Samples

Shimadzu's Nexera UC system enables highly reliable and stable analysis of delicate samples that are prone to oxidation or dissociation if exposed to air. Notably, in the analysis of pesticides in food products, the system takes only five minutes for a complete analysis sample pre-treatment when compared with at least 35 minutes for conventional systems. Furthermore, the fully automated system has a particularly high target analyte recovery rate and reduces the possibility of human error during analysis. The system offers a wide range of separation modes, enabling the separation of a diverse wide range of compounds at once, which is not possible with single systems that are based on gas and liquid chromatography.

Shimadzu Corporation
www.shimadzu.com

Breaching Overcome limits with Berghof high-pressure reactors

The modular design and configuration options of Berghof Highpreactor high-pressure reactors are a response to the requirements of modern synthesis laboratories. Safety, reliability and economy are achieved through the use of high-quality materials combined with durable PTFE linings. Due to its chemical resistance PTFE is especially suitable for use with highly corrosive media. Thick-walled PTFE lining and inserts provide protection for all wetted components. The use of special cost-intensive alloys like Hastelloy is therefore not necessary. In addition, handling is simplified by the use of quick-lock chains and exchangeable valves. To make laboratory work easier, a large range of capacities from 25ml to 4l are available, with various stirring and heating technologies, as well as temperature regulators and data loggers.

Berghof
www.berghof.com

IMPRINT

Published by
Wiley-VCH Verlag GmbH & Co. KGaA
A Company of John Wiley & Sons, Inc
GIT VERLAG
Boschstr. 12, 69469 Weinheim, Germany
Tel.: +49 6201 606 0

Geschäftsführer
Dr. Jon Walmsley, Sabine Steinbach

Publishing Director
Roy Opie

Director Corporate Sales PSE
Dr. Katja Habermüller

Editorial Director International Publications Laboratory & Biotechnology
Dr. Martin Friedrich

Editor in Chief
Dr. Arne Kusserow
Tel.: +49 6201 606 732 · Fax: -793
arne.kusserow@wiley.com

Editors
Dr. Anja Gaugel
Tel.: +49 6201 606 716 · Fax: -793
anja.gaugel@wiley.com

Dr. Martin Graf-Utzmann
Tel.: +49 6201 606 766
martin.graf-utzmann@wiley.com

Dr. Birgit Washburn
Tel.: +49 6201 606 760· Fax: -793
birgit.washburn@wiley.com

Editorial Assistant
Tina Weber
Tel.: +49 6201 606 751 · Fax: -793
tina.weber@wiley.com

Editorial Assistant
Yadigar Manav
Tel.: +49 6201 606 752
yadigar.manav@wiley.com

Segment Sales Manager Laboratory & Biotechnology
Vanessa Winde
Tel.: +49 6201 606 721
vanessa.winde@wiley.com

Media Consultants
Dipl.-Ing. Oliver Gerber
Tel.: +49 6201 606 717 · Fax: -793
oliver.gerber@wiley.com

Dr. Stefanie Krauth
Tel.: +49 6201 606 728 · Fax: -793
stefanie.krauth@wiley.com

Bettina Willnow
Tel.: +49 6201 606 770 · Fax: -793
bettina.willnow@wiley.com

Dipl.-Betriebsw. Andreas Zimmer
Tel.: +49 6201 606 763 · Fax: -793
andreas.zimmer@wiley.com

Sales Representative
Dr. Michael Leising
Tel.: +49 6131 88 39 22 · Fax: -11
leising@leising-marketing.de

Sales Administrator
Kerstin Kunkel
Tel.: +49 6201 606 731 · Fax: -790
kerstin.kunkel@wiley.com

Wiley GIT Customer Service
65341 Eltville / Germany
Phone: +49 6123 9238 246
Fax: +49 6123 9238 244
E-Mail: WileyGIT@vuservice.de
Our service is available for you from Monday to Friday 8 am – 5 pm CET

Production
Wiley-VCH Verlag GmbH & Co. KGaA
A Company of John Wiley & Sons, Inc
GIT VERLAG

Christiane Potthast
Tel.: +49 6201 606 741 · Fax: -793
christiane.potthast@wiley.com

Layout/Litho
Oliver Haja (Layout)
Ramona Kreimes (Litho)
Elli Palzer (Coverpage)

Reprints
Dr. Stefanie Krauth
Tel.: +49 6201 606 728
stefanie.krauth@wiley.com

Printed by
pva, Druck und Medien,
Landau, Germany

Circulation
20,000 copies, 6 times per annum
Advertising price list from
October 1st 2014

Subscription 2015
Six issues € 77.00, single copy € 15.40 plus postage. Pupils and students receive a discount of 50% at sight of a valid certificate. Subscription orders can be revoked within one week in writing. Dispatch complaints are possible only within four weeks after publishing date. Subscription cancellations are accepted six weeks before end of year.

Bank account
Commerzbank AG, Mannheim, Germany
Account No. 07 511 188 00
Routing No. 670 800 50
BIC: DRESDEFF670
IBAN: DE94 6708 0050 0751 1188 00

Printed in Germany
ISSN 1611-6038
www.gitverlag.com

OLYMPUS

Your Vision, Our Future

cellTIRF

The next level of TIRF microscopy.

Olympus cellTIRF provides advanced TIRFM solutions, with a series of peerless features such as highly advanced optics, unique independent laser control and exceptional accuracy.

· Easy to use for TIRFM, HILO, and FRAP
· Software adjustment of penetration depths.
· Advanced TIRFM objectives family
· Broad range of cell* family lasers

See more at **www.olympus-lifescience.com**

OLYMPUS EUROPA SE & CO. KG
Postbox 10 49 08, 20034 Hamburg, Germany | Tel. +49-40-237-730 | www.olympus-europa.com