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New NMR Methods for Reaction Monitoring

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Nuclear magnetic resonance spectroscopy (NMR) is used to study the structure of molecules, the interaction of various molecules, the kinetics or dynamics of molecules, as well as the composition of mixtures of biological or synthetic solutions or composites.

The size of the molecules analyzed can range from a small organic molecule or metabolite to a mid-sized peptide or a natural product, all the way up to proteins of several 10s of kDa in molecular weight.

Nuclear Magnetic Spectroscopy (NMR) complements other structural and analytical techniques such as X-ray, crystallography and mass spectrometry. NMR's advantage is the unique ability of a nuclear spectrometer to allow both the non-destructive and the quantitative study of molecules in solution and solid state, as well as to enable the study of biological fluids.

NMR is becoming an increasingly important tool for the monitoring of chemical reactions. Utilization of the inherent structural and quantitative information delivered via NMR spectroscopy can provide detailed mechanistic insights and increase process understanding. Previously, implementation of online reaction monitoring by NMR had been held back by tedious experiment setup and the need for manual or offline data processing and analysis. However, that is not the case any longer.

Bruker Biospin's InsightMR brings real-time monitoring of chemical reactions by NMR to the lab, with integrated acquisition control and automatic data processing and analysis.

From the lab to the plant

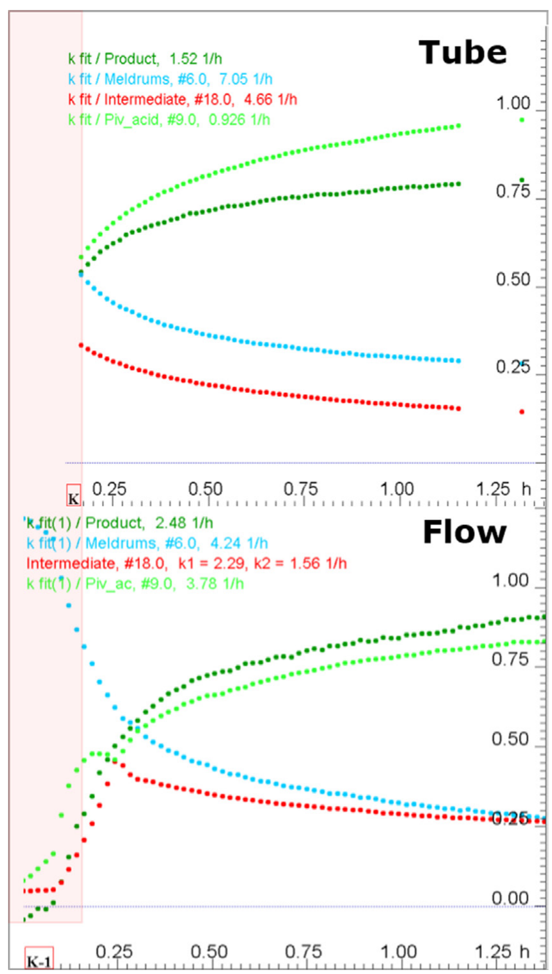
In an effort to understand reaction mechanism, a team, which included Bruker's Director of Pharmaceutical Business Unit, Anna Codina, compared reaction monitoring using NMR versus vibrational spectroscopy. For this experiment, the reaction was monitored in an NMR tube. Resulting data showed the profiles were very similar, and the two techniques were in agreement, but they were not identical. Regardless, there was enough data to demonstrate that NMR complements vibrational spectroscopy, enhancing reaction understanding so that processes can be transfered from the lab to the plant with a high degree of confidence.

However, the research team wanted to take their study a step further, forging a collaboration with colleagues at Pfizer. The same combination of techniques was used, but this time, the reaction was monitored with a sample flow tube.

The researchers ran the reaction outside of an NMR tube, in a reactor, in real conditions. They then pumped out the reaction mixture from the vessel to the NMR to acquire the data. At this point, the reaction went back in a temperature-regulated closed loop.

Resulting data indicated running the experiment by flow rather than tube allowed the ability to better capture the start of the reaction. With tube NMR, the first 10 minutes of the reaction was missed—something that was not a problem with flow NMR. Flow NMR also mimics real reaction conditions in terms of temperature and pressure, and enables the simultaneous acquisition of IR, pH and mass spec.

Flow NMR vs. Tube NMR



The combined use of NMR and infrared leads to strategic process decisions and ultimately cost savings, tighter control and consistent products.

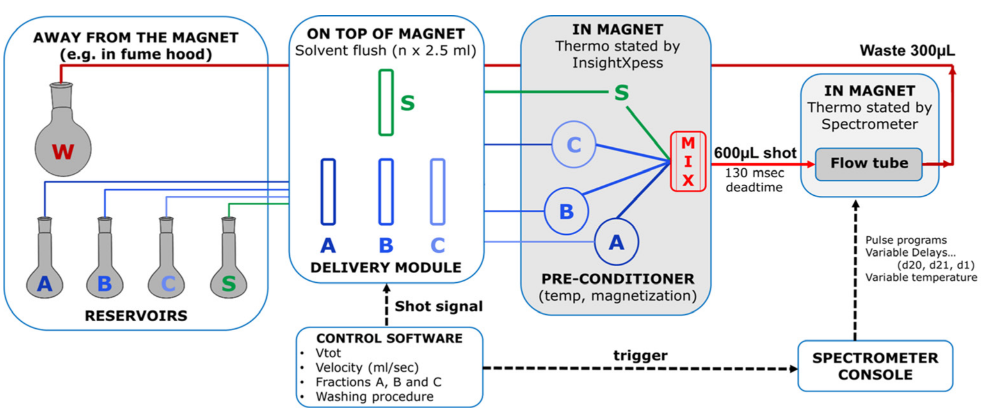
Fast, very fast

While using Bruker's InsightMR for the above experiments worked well, depending on experimental conditions, it may not always be the solution. For example, some situations call for faster monitoring. For those cases, Bruker pioneered InsightXpress, the fastest of the InsightMR family, which enables monitoring of fast reactions and rapid, automated screening of reaction conditions.

InsightXpress moves the reaction inside the NMR spectrometer. It is a combination of the flow unit, high speed delivery pump, and the NMR spectrometer itself. Schematically, the reservoirs—including the samples, solvent and waste bottle—are held away from the magnet, in a fume hood for example. On top of the magnet is the delivery module, which allows injection into the system (and also includes a flush line). In the magnet, there is the pre-conditioner, which allows InsightXpress to control temperature and magnetization of the reagents.

The system is all connected by tubing. The software controls total volume, reagent ratios and velocity of the injection. A small volume (600 µL or less) is injected into the flow tube to be detected. After the injection, a short trigger signal is generated by the delivery module to coordinate NMR acquisition. Essentially, there is communication between the delivery module and the console that is waiting to acquire data.

A schematic of Bruker's InsightXpress.



The Suzuki-Miyaura coupling reaction was used as an example reaction for InsightXpress as it is a popular and very fast reaction. It is a palladium-catalyzed bond-forming reaction, coupling a boronic acid with an electrophile. The boronic acids can protodeboronate, reducing yields and efficiencies of the cross-coupling. In the case of polyfluorophenyl boronic acids, this is extremely fast and renders the monitoring of the cross-coupling by traditional NMR techniques impracticable.

For this test, researchers compared manual sample preparation with the in-situ stop flow monitoring using InsightXpress. They wanted to measure 14 observed rate constant (Kobs) for 20 data points. Manually this would require 280 NMR samples for a total of two weeks of work for a chemist. But the researchers were able to obtain the same information using much less chemicals and only 3 hours of work using InsighXpress on a Bruker's platform.

This allowed the researchers to make several conclusions: 1) stop flow (SF) techniques allow fast, reproducible monitoring of fast reactions, with accurate temperature control; 2) SF-NMR and SF-IR allow a complete mechanistic study of the protolysis of polyfluorophenyl boronic acids; and 3) fast deboronations proposed occur via a stabilized aryl anion, due to the inductive influence of the fluorines.

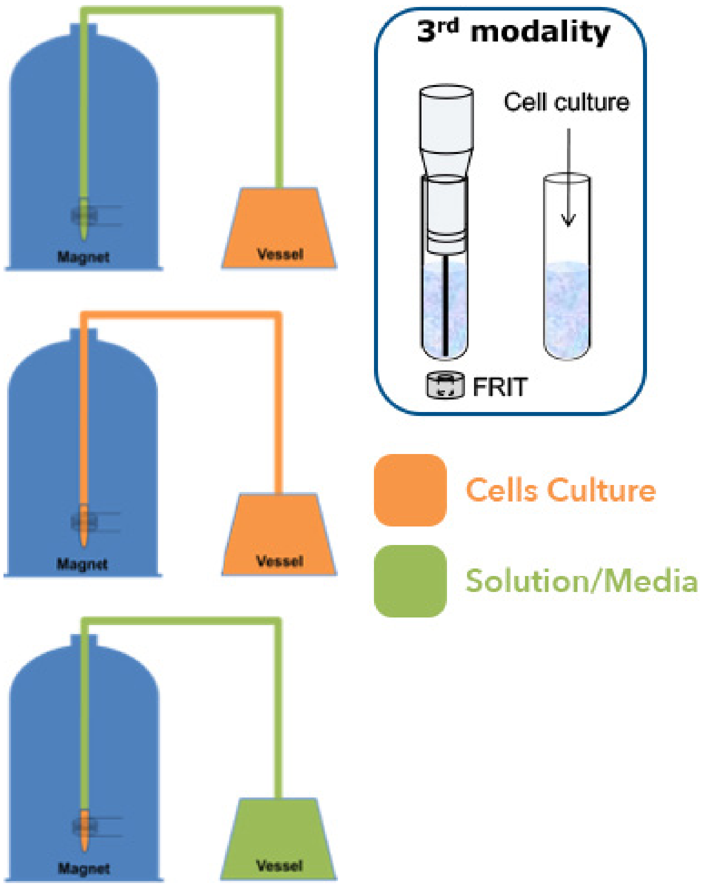
Biological activity

Once the monitoring of very fast reactions had been tackled, Bruker set their sights on monitoring biological cell activity. InsightCell, another arm of the InsightMR portfolio, can provide insight into cell activity with real-time NMR monitoring. It features a flow unit that is similar to InsightMR, a NMR tube and temperature-controlled transfer line. This can be combined with a temperature control unit and a pump system to transfer reactions from a bioreactor, enabling researchers to obtain unique structural information about compounds consumed and produced by cells.

There are three possible modes of operation to study the activity of living cells by NMR: 1) flow only the solution to the NMR tube, keeping the cells culture away from the magnet, in the bioreactor; 2) flow the whole suspension through the flow unit; or 3) trap the cells line in the NMR tube only.

For an example of the third modality, Bruker scientists collaborated with a team at the University of Birmingham to monitor lymphophatic leukemia cells.

With InsightCell, there are three modalities available to study living cells.



The researchers were able to monitor the lymphatic leukemia cells using the third modality, or keeping the cells inside the NMR tube only. They put the cell culture into the NMR tube, used a 5µ pore size frit to keep the cells, and used a flow rate to maintain the cells on suspension. They combined InsightCell with an incubator to control temperature, carbon dioxide and oxygen levels. They monitored cell activity for a total time of eight hours. The team was able to detect signals from more than 30 extracellular metabolites.

Analysis of time course data showed a significant decrease in glucose and increase in lactate over time, corresponding to the Warburg effect. This can be in the cancer cell line, produced by a high rate of glycolysis followed by lactic acid fermentation. In this case, NMR could be used to monitor this activity and increase understanding of the Warburg phenomenon in general.

Conclusion

As demonstrated by multiple case studies, Bruker Biospin's InsightMR flow solutions bring real-time monitoring of chemical reactions by NMR to your lab, with integrated acquisition control and automatic data processing and analysis.

The classic laboratory setup when using InsightMR for chemical reaction.

InsightMR has a varied portfolio of applications for monitoring chemical and biological processes. InsightMR enhances reaction understanding and confidence when transferring processes from the lab to the manufacturing plant, while InsightXpress enables the monitoring of very fast reactions. InsightCell monitors living cells to study their biological activity through direct measurement of variations in metabolite concentration.

Equipped with intuitive, easy-to-use software, the InsightMR platform is designed for both NMR experts and non-experts when straightforward monitoring of reactions is necessary.

> "NMR is becoming an increasingly important tool for the monitoring of chemical reactions."

ABOUT THIS TECHNICAL PAPER

This white paper is adapted from a webinar sponsored by Bruker Corporation and presented by Advantage Business Marketing.

Two representatives from Bruker BioSpin were the speakers, and Advantage Business Marketing Editor-in-Chief Michelle Taylor, was the moderator.

Anna Codina, Ph.D., Director, Pharmaceutical Business Unit at Bruker BioSpin is responsible for the NMR pharma market at the company, and manages a portfolio of products designed to suit the needs of the pharmaceutical industry. Before joining Bruker, Anna worked for several pharmaceutical companies (Pfizer, Genentech and Pharmhispania), which allowed her to develop skills in drug development, analytical chemistry, structure elucidation and reaction monitoring. During her post-doc, Anna studied protein binding by NMR at the LMB-MRC, Cambridge UK. She obtain her Ph.D. from the University of Barcelona, Spain on protein NMR.

Matteo Pennestri, Ph.D., is the product manager for InsightXpress and InsightCell at Bruker BioSpin. He is also an applications scientist in liquid state NMR. Before joining Bruker, he was research fellow at Trinity College in Dublin, Ireland. Matteo was previously an applications scientist at Agilent Technologies (formerly Varian) for the EMEIA region. He also worked as an NMR spectroscopist for the pharmaceutical companies Merck Sharp and Dohme and EISAI, where he was responsible for NMR support for medicinal chemistry. He received his Ph.D. from the University of Roma, Italy in Biophysics, solving 3-D structures of proteins and investigating protein-ligand interactions by NMR.

Michelle Taylor is the Editor-in-Chief of Laboratory Equipment and Forensic Magazine, with both brands a part of Advantage Business Marketing. Michelle has held a variety of roles within Laboratory Equipment in her eight years with the company, most recently being promoted to Editor-in-Chief in 2014. She is responsible for managing the day-to-day operations of the brand, as well as overseeing the content and production of all issues and related websites. She writes in-depth feature articles, industry news, blogs and reports. Michelle earned her B.A. in Journalism from Elon University in Elon, N.C.

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