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November 2009 ›››

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Fundamental Research in Chemistry and Biochemistry

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Chemistry is the foundation of our material world, and understanding the fundamentals of chemistry unlocks our ability to develop new sources of energy, create new and advanced materials, and ensure we maintain a sustainable relationship with the environment. Biochemistry and life sciences take that knowledge a step further, towards understanding the fundamentals and mechanisms of life itself – permitting us to develop life-saving medications and enhancing the ability of our planet to sustain life.

As we progress in our fundamental knowledge and make new discoveries, it does not seem we ever reach an endpoint in our research. We continue to discover new areas of exploration, and, as we do so, we continually need new and better ways of measuring the results of our research – to ensure our results are as accurate and informative as possible.

METTLER TOLEDO has long been involved in all fields of scientific research, with measurement tools of the highest quality. Our contributions to the advancement of research in chemistry and biochemistry are felt in almost every laboratory around the world. Below we highlight some of the more recent, exciting results and research methods.

PARALLEL SYNTHESIS & PURIFICATION OF PEPTIDE LIBRARIES

Based on the publications of Anthony G. M. Barrett et al., Imperial College London[1]

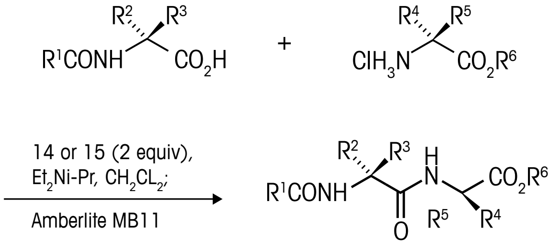

Figure 1.

A major desire for chemists working in drug discovery is to synthesize libraries of pure compounds without the headaches and bottlenecks created by downstream work-up and purification procedures. The peptide coupling reactions as shown in Figure 1 were performed using functionalized reagents followed by solid phase extraction. Both purification issues related to this reaction are eliminated by simple filtration followed by solid-phase extraction.

The key is having both coupling reagents functionalized to a solid support. Since both reagents are used in excess, they are easily removed by filtration. The synthesis begins by suspending the functionalized reagents into dichloromethane and allowing them to react with ethyldiisopropylamine containing the n-protected amino acid and the amino ester using a METTLER TOLEDO Miniblock®. The coupling reactions take place for 12 hours at 25°C.

Excess reagents are removed by filtration via transfer into a second MiniBlock® loaded with ion exchange resin. The reaction mixture was in contact with the ion exchange resin for 2 hours, allowing the salt by-products (as formed by the reaction) to remain bound to the resin. The pure peptides were then collected into a deep well plate and evaporated.

	18	19	ROMP-sphere	% Yeild[a]	% Purity[b]
1	Z-Gly	(L)-Phe-OEt · HCl	14	96	95[c]
2	Z-Gly	(L)-Phe-OEt · HCl	15	86	87[c]
3	Boc-Aib	Gly-OEt · HCl	14	97	98
4	Z-Gly	Aib-OMe · HCl	14	97	98
5	Z-Gly	Aib-OMe · HCl	15	84	96
6	Boc-Aib	Aib-OMe · HCl	14	90	98
7	Z-(L)-Leu	Gly-OEt · HCl	14	89	92[d]
8	Z-(L)-Leu	Gly-OEt · HCl	15	92	94[d]
9	Z-Gly (L)-Phe	(L)-Val-OMe · HCl	14	95	90[c]
10	Z-Gly (L)-Phe	(L)-Val-OMe · HCl	15	87	87[c]
11	*N*-Fmoc-Aib	Gly-OEt · HCl	15	85	88
12	*N*-Fmoc-Aib	(L)-Ala-OEt · HCl	15	80	89[d]

a Isolated yields
b Purity as determined by 1H NMR
c Product completely racemized (epimerized)
d Extent of racemization not determined

Table 1. Reaction reported to be excellent for coupling hindered amino acids. Average % yields are 90% and % purities are 92%. Entry 6, unnatural amino acid AIB coupled with a 90% yield and 98% purity.

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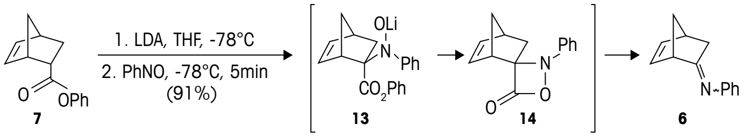
[1] Facile and purification free synthesis of peptides utilizing ROMPgel and ROMPsphere-supported coupling reagents. Barrett, Bibal, Hopkins, Koebberling, Love, and Tedeschi. Department of Chemistry, Imperial College London. Tetrahedron 61 (2005) 12033–12041

NITROSOBENZENE-MEDIATED C–C BOND CLEAVAGE REACTIONS AND SPECTRAL OBSERVATION OF AN OXAZETIDIN-4-ONE RING SYSTEM

Payette, J. N.; Yamamoto, H. J. Am. Chem. Soc.; (Communication); 2008; 130(37); 12276-12278. DOI: 10.1021/ja804325f

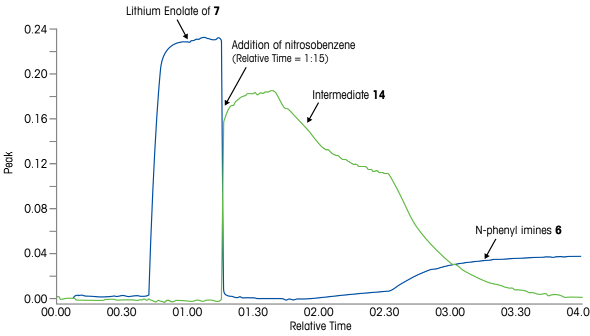


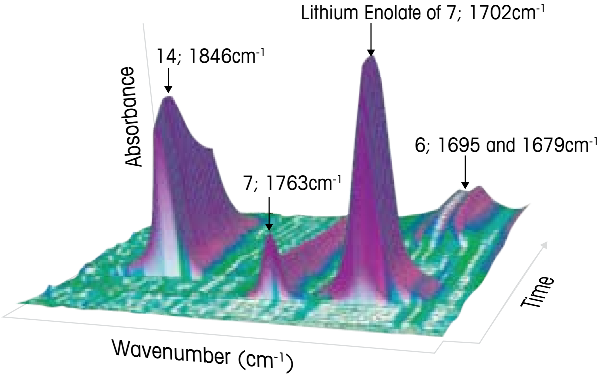
Note: The intensity of the line plot of 6 is reflective of only one isomer of 6.

ReactIR™ allowed the reaction under investigation to be characterized and the reaction pathway confirmed using the high molecular specificity of the mid-infrared spectrum. Offline analysis was not possible due to the low temperature (-78°C) requirement.

3D and 2D plots of ReactIR™ experimental data show that the decarboxylation reaction actually follows a deprotonation/intramolecular trans-esterification/fragmentation sequence (more detail given in the publication) with the first spectral observation of an oxazetidin-4-one heterocycle 14.

Immediately after addition of nitrosobenzene to the lithium enolate of 7 at -78°C, oxazetidin-4-one 14, having an IR stretch at 1846 cm^{-1}, is observed. As the cooling bath is gradually warmed, the disappearance of 14 is marked by the appearance of N-phenylimines 6.





ReactIR™ leads the way in real-time *in situ* reaction analysis

ReactIR™ is used by the world’s top researchers to increase the understanding of organic chemistry. ReactIR™ monitors reactive chemistry using well understood mid-infrared spectroscopy. A robust ATR probe is inserted directly into the reaction vessel, providing a “molecular video” of the reaction. The concentration changes of all key reactive and transient species are monitored allowing for mechanism and pathway determination. Accurate kinetics analysis is easily achieved with automated collection of a comprehensive data set.

With over 150 academic references in the published literature and widespread use in industry, ReactIR™ is an acknowledged leader in elucidation of reaction kinetics and mechanisms.

The study of chemistry under actual conditions from highly concentrated to sub-millimolar eliminates common errors associated with offline sampling and analysis. ReactIR™ measures *in situ* to ensure reactions sensitive to moisture, temperature or oxygen can be easily characterized without disturbing the reaction or compromising the chemistry.

ReactIR™ with iC IR™ software makes it simple to collect, visualize, interpret and report results. iC IR™ helps scientists distill information-rich data into valuable chemistry understanding through the utilization of chemometrics that identify and characterize reaction components. This allows users to gain valuable information without extensive spectroscopy knowledge or training.

Most Active Research Areas for ReactIR™:

- Organometallics and catalysis
- Biocatalysis
- Organic synthesis
- Polymers
- Mechanism and theory
- Green chemistry

Chemical Reaction Engineering: Chemistry on a Grand Scale

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Chemical engineers are responsible for transferring fundamental knowledge into practical solutions for producing the chemical products we use every day. Whether it is the processing and production of fuel, plastics, food, paper, shampoo, or pharmaceuticals – the chemical engineer helps deliver a product that meets the needs of the end user, while ensuring safety, efficiency, and economy in the manufacturing process.

Ensuring safety, efficiency and economic profitability in a chemical process is a balancing act of applying knowledge of physical laws, rules of thumb, personal and corporate priorities, and government regulations. It is not a simple feat, but it can be achieved with thorough research and design methods. Fortunately, as regulatory and economic constraints tighten, advancements in measurement technology and improvements in process understanding allow for more robust engineering designs with tighter operating tolerances and greater reliability.

Accurate reactor control and reliable *in situ* process monitoring are basic requirements for ensuring a chemical process reliably meets the production goals for yield and quality. METTLER TOLEDO, with automated laboratory reactors outfitted with real-time calorimetry and *in situ* mid-IR spectroscopy, provides tools for fully characterizing the heat and material balance of chemical reactions. This fundamental information is vital for cutting-edge work incorporating process design, process control and process safety in the holistic modeling, optimization, and scale-up of chemical reactions.

RTCal™ vessel with integrated heat flux sensors.

FASTER DETERMINATION AND CONFIRMATION OF THERMODYNAMIC MODELS

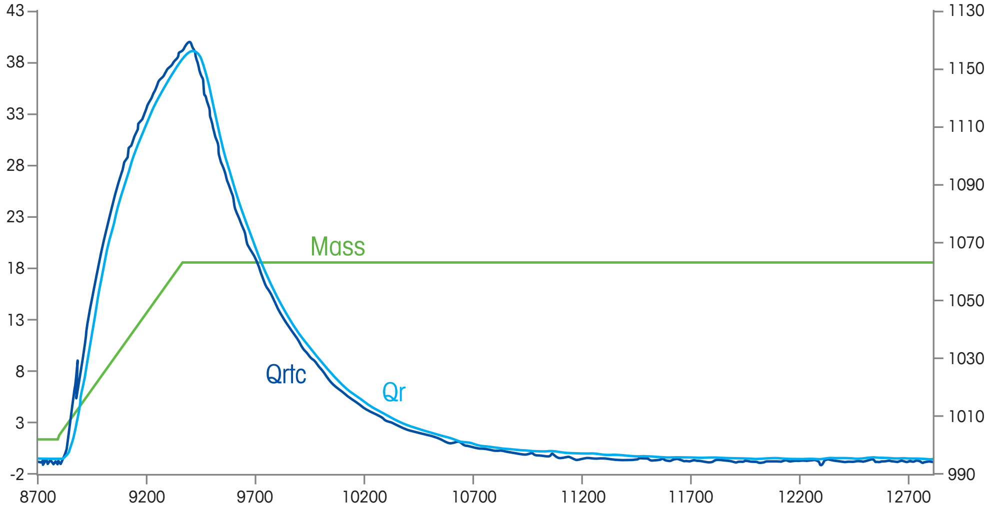
Accurate measurement of the heat of reaction and the thermal properties of the reaction mass is necessary for effective process modeling and design of a scalable reaction.

RTCal™ is a unique method of measuring heat flow in real time, extending the application of real-time calorimetry to investigate complex processes without requiring calibration during each experiment. RTCal™ technology is based on heat flux sensors located within the jacket of a double-walled reactor vessel. Vertically and horizontally positioned sensor bands detect the heat flow across the wall of the reactor online and in real time.

RTCal™ provides accurate heat data under isothermal and non-isothermal conditions. Using RTCal™ the heat transfer coefficient is computed in real time during the experiment; the specific heat of the reaction mass is provided automatically with every temperature ramp.

The heat flow information is subsequently processed, and can be used to compute the overall heat of reaction including heats of dosing and accumulation.

Experiments with RTCal™ are independent of the properties or the behavior of the reaction mass. Hence, no calibration procedures need to be run before, during or after the reaction. The enhanced set of information allows more detailed conclusions and full confidence in the experimental results.



RTCal™ provides accurate calorimetry information in real time, for improved process modeling and design

PROCESS SAFETY ANALYSIS AND SCALE-UP OF EXOTHERMIC REACTIONS

WeylChem Corporation, a global fine chemical company specializing in custom synthesis, performed experiments to develop a protocol for a novel synthesis of 2,4-diformylmesitylene (DFM) that would be scalable to production. A key reactant (potassium 2-nitropropane) is known to self detonate, which requires WeylChem to produce and use the salt *in situ*. Understanding the energy involved with accumulation and reaction of this salt in the reactor was important in avoiding a potential runaway reaction incident.

An initial set of only three experiments was carried out in a METTLER TOLEDO RC1e® reaction calorimeter using different addition rates to assess the impact of feed rate on the heat of reaction, thermal accumulation and maximum temperature rise with cooling failure (TCF) as illustrated in the figures below.

WeylChem's experience: in their own words

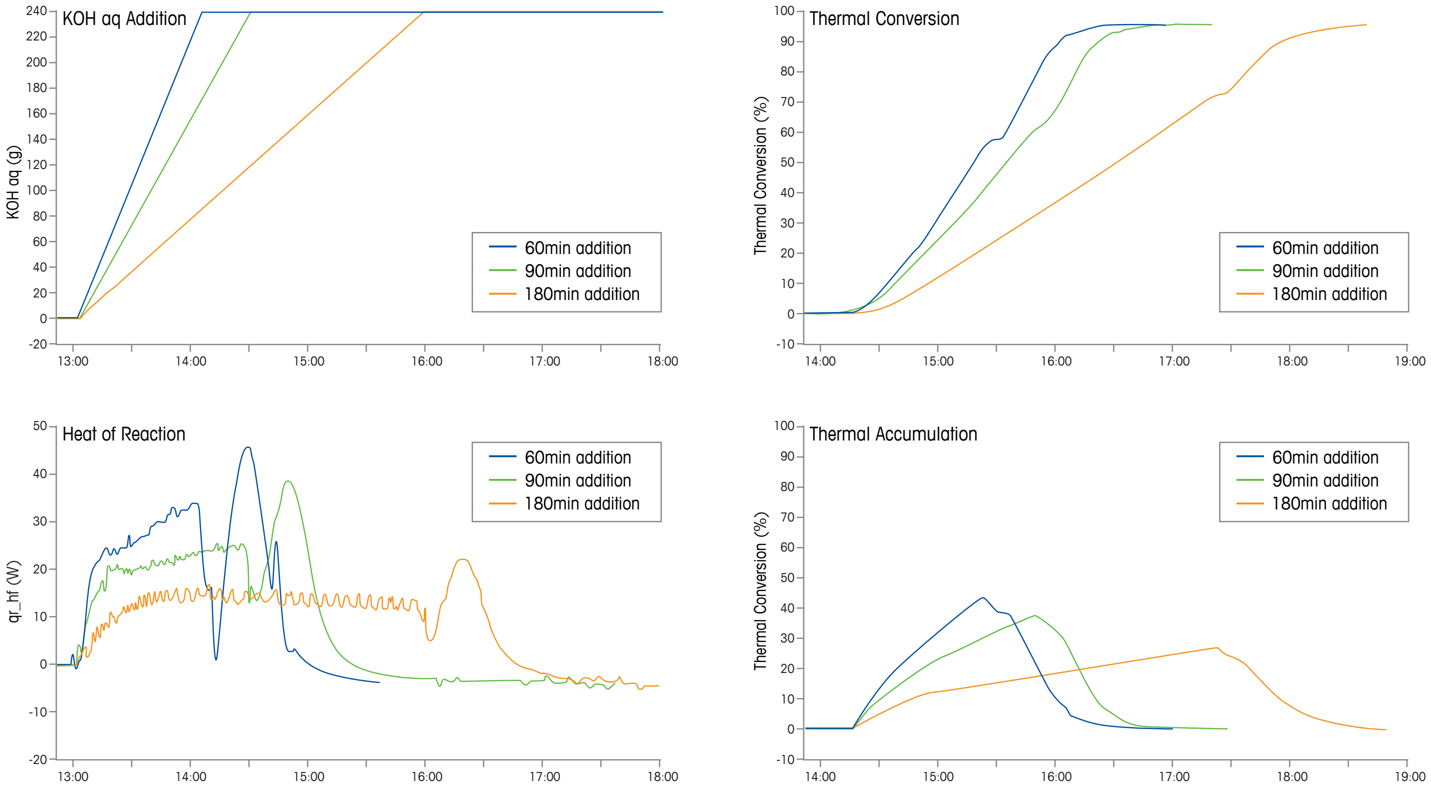
From only three experimental runs we were able to get very useful data from the new iC Safety™ software. This data has provided good insight into the heat of the reaction as well as the amount of heat generated during different portions of the reaction. Without the use of the software in conjunction with the RC1e® we would not have discovered how exothermic this reaction was until we scaled up the reaction in the plant. (Lab runs did not show any heat kick or large exotherm.) We found that the amount of heat generated will preclude us from doing the fast feed that we had envisioned.

> **"...a safer reaction that has a better chance of producing the desired results..."**
>
> – WeylChem

With the three different feed rates and subsequent heat ups, we were able to obtain enough data to allow us to extrapolate cycle times, run-away scenarios and cooling failure issues.

From the data that we accumulated, we found that we will need a minimum of 120-180 minutes for the feed (heat removal) and possibly a slower heat-up rate to the digest temperature to help in the overall heat removal. From this study, in conjunction with additional DSC data, we now feel confident that we have developed a protocol that will allow for the scale-up of this reaction to a plant production scale in the future. In addition, this added information allowed for a more accurate costing on this desired intermediate molecule.

The reaction's heat profile was only one area that the software helped elucidate. In addition, it helped with possible quality issues as they related to safety yields. By this I mean, the ability to know what temperature the pot would be at any point during the addition if we lost cooling, which allows us to set up a safer reaction that has a better chance of producing the desired results even if we had a cooling failure during the reaction.



iControl™ and iC Safety™ simplify and speed up thermal hazard analysis. Results showing the affect of (a) KOH solution addition on (b) heat of reaction, (c) thermal conversion, and (d) thermal accumulation.

Particles and Droplets: Research in Multi-Phase Systems

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Particles and droplets are virtually everywhere in the chemical, consumer products, and biopharmaceutical industries. Particles grow, agglomerate, break, dissolve, and change shape – all of which can have an impact on process performance and product quality. To successfully work with multiphase processes, scientists and engineers must fully understand and optimize the particle distributions to control process efficiency such as filtration rates, flow properties, or dissolution rates. In addition, particle distributions must be well controlled to ensure repeatable product properties such as yield, purity, and bulk density.

An understanding of how particles and droplets exist within the actual process environment – at full process concentration and under standard operating conditions – is necessary for proper design of the processes and equipment that can handle those particle and droplet systems effectively. Sampling and dilution of particle systems is fraught with difficulties and almost always has measurable impact on the particle system itself.

METTLER TOLEDO FBRM® and PVM® technologies enable scientists and engineers to measure real-time changes in particle count, dimension and shape. In many cases, these inline measurements lead to insights into the particle systems that are not possible with traditional offline measurements, and permit the researchers to model and develop more effective processes and equipment for the consistent production, separations, and handling of particle and droplet systems.

PARTICLE AND DROPLET RESEARCH FOR PETROLEUM RECOVERY

Understanding Gas Hydrates, Asphaltenes, Oil Suspensions and Emulsions

There are several flow assurance problems that occur during oil production, recovery, and transport. In cases of extreme temperatures and pressures, it is possible that methane gas hydrates crystallize or asphaltenes precipitate in the pipeline. If not properly controlled, the crystals or particles may agglomerate to the point of plugging the pipeline. This can be costly as deposition or blockage may damage the reservoir and affect the oil production rate. Therefore, it is critical to study how additives may be used to better understand, optimize, and control agglomeration and precipitation.

Also in the case of oil recovery and transport, water is used to displace oil in the well. Ultimately, the water and particulates must be removed from the oil, whether by physical or chemical methods. Therefore it is necessary to quantify how much water or particulate remains trapped in the oil before and after the unit operation. For example, de-emulsifying studies are conducted with FBRM® droplet analysis to improve the separation process of oil and water, by varying the type and concentration of different additives.

Particles and Droplets Measured Directly in Raw Crude Oil

Probe-based tools are ideal for characterizing methane gas hydrates, asphaltene particles, wax precipitates, and oil emulsions. In most cases, the conditions (high pressure) to which hydrate crystals form and asphaltenes precipitate make it difficult to sample. The particle and droplet systems are extremely sensitive to temperature and pressure fluctuations.

Offline techniques require samples that are dilute enough to allow for simple measurement. However, the measurement of dilute samples is not representative of the actual system and provides a limited data set. With FBRM® and PVM® probes the user can track the entire dynamic particle and droplet system in crude oil at standard operating temperatures and pressures, without sampling or sample dilution.

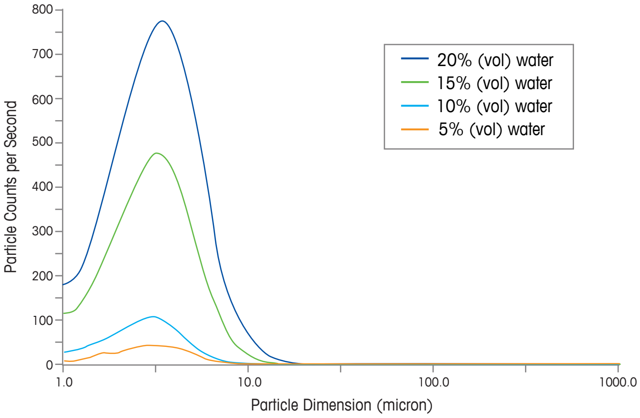

Figure 1. Studying the formation of crude oil emulsions and crude oil emulsion stability. FBRM® is used to characterize and quantify the droplet population and dimension of water droplets in crude oil at different concentrations – without sampling or dilution. The researcher can quantify the effects of water concentration on droplet population and size, monitor the effectiveness in various additives in breaking down the emulsion, and characterize the efficiency with which water is separated from oil in a hydrocyclone.

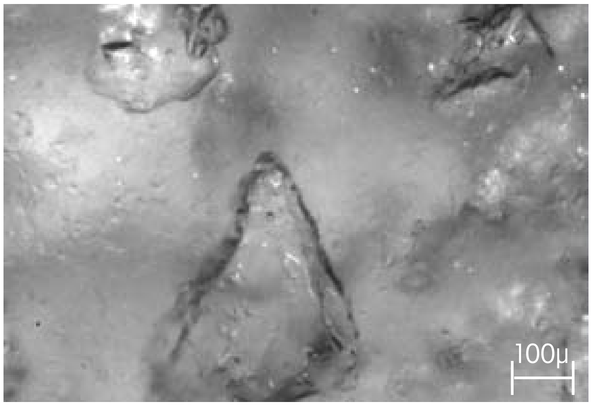

Figure 2. Hydrate crystal formation detected in undiluted crude oil. Measured in high-pressure research vessel using PVM® inline imaging.

ANTISOLVENT CRYSTALLIZATION IN-PROCESS CHARACTERIZATION

Des O'Grady, Mark Barrett, Brian Glennon
University College Dublin (UCD)

In-process tools can provide a deeper understanding and allow for faster characterization and development of crystallization processes. Effective implementation of in-process tools allows crystallization processes to be monitored in real time with no need for sampling. Sampling can be time consuming and nonrepresentative especially for crystallization systems where samples can change due to nucleation, growth, agglomeration and/or breakage once removed from the crystallization vessel. In-process tools may also be implemented at a range of scales making it possible to compare crystallization experiments performed in the laboratory with batches run in the pilot-plant or at the full production scale. This is especially important for antisolvent systems where changes in mixing conditions between scales can make effective scale-up, and subsequent optimization of manufacturing processes, difficult.

In this study, the impact of antisolvent addition rate on crystal size and morphology is assessed through experiments based on the unseeded crystallization of benzoic acid from ethanol-water mixtures using water as the antisolvent. The results of two experiments are presented in a summarized form, one at 'fast' antisolvent addition rate – 0.2 gs^{-1} – and one at 'slow' antisolvent addition rate – 0.1 gs^{-1}. Supersaturation, monitored using ReactIR™, was approximately twice as high for the fast addition rate compared to the slow addition rate. FBRM® distributions indicate that a fast addition rate produces significantly smaller crystals compared to the slow addition rate. PVM® images confirm these findings and show that the crystals tend to be finer needleshaped crystals with significant agglomeration at the faster addition rate.

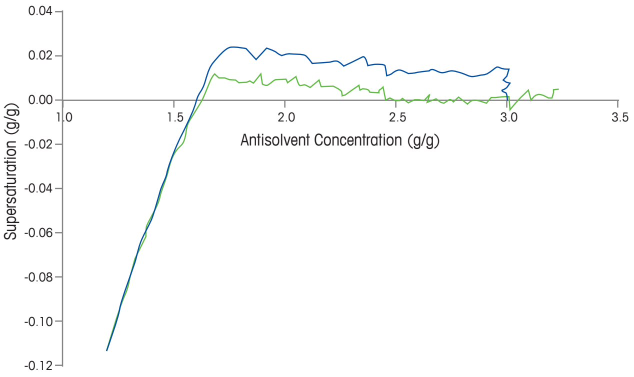

Figure 1. Supersaturation monitored in real time with ReactIR™

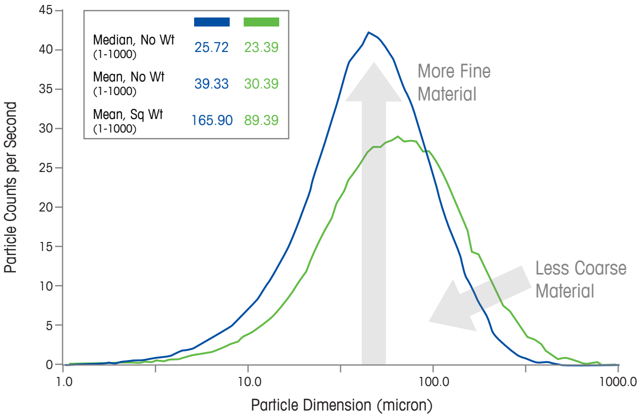

Figure 2. FBRM® distributions at both addition rates (0.1gs^{-1} – blue; 0.2gs^{-1} – green)

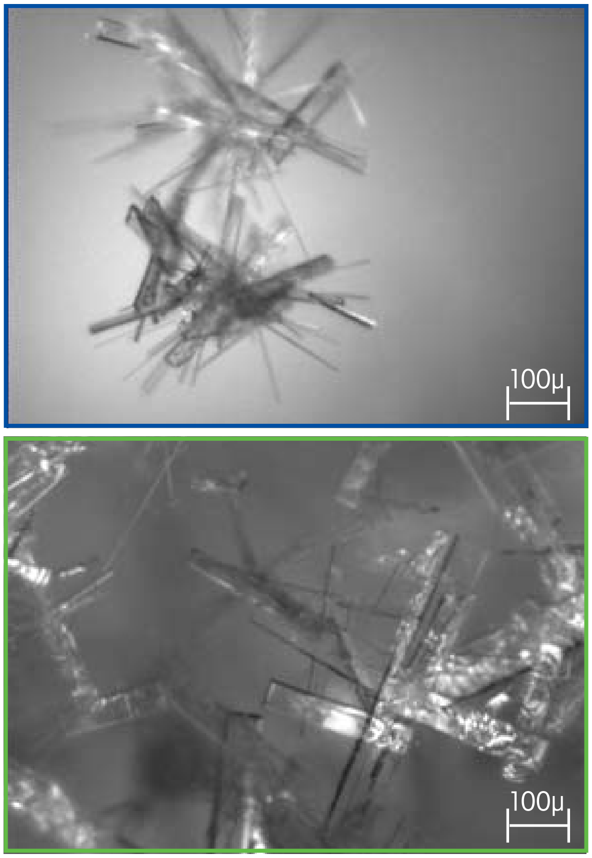

Figure 3. PVM® images at both addition rates (0.1gs^{-1} – blue; 0.2gs^{-1} – green)

Webinar Spotlight: In-process monitoring of particles and droplets in emulsions, suspensions, and multiphase flow

Emulsions, suspensions, and multiphase flows present several challenges for reliable particle and droplet characterization. Due to process conditions, it may be difficult or unsafe to sample. The physical properties of the suspension or emulsion may be altered from dilution or handling.

This webinar focuses on diverse applications in emulsions, suspensions, and multiphase flows, with Focused Beam Reflectance Measurement (FBRM®) and Process Video Microscopy (PVM®) technologies to characterize multicomponent liquid and/or solid phases in-process and in real time.

Case Studies

Application examples presented in this webinar using both in-process technologies include:

- Liquid-liquid emulsions
- Crude oil and water emulsions
- Asphaltene precipitation
- Emulsion and suspension polymerization

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- Particle System Characterization
- Pharmaceutical Product Engineering
- Mechanisms of Dissolution and Disintegration

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