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XRD APPLICATION NOTE

On-line crystallization monitoring

Slurry Flow Cell for *in situ* investigation of crystallization processes

Introduction
The basics of crystallization, such as the fundamentals of thermodynamics, kinetics, and structure, are well established. However, the application of these basics to solve crystallization process problems is complicated with a large number of tools for the practicing scientist or engineer to make use of. Control of crystal size distributions, polymorphs, impurity-crystal interactions, and morphology (shape) are a few of the key areas in current research with multiple approaches under evaluation. Most approaches are targeted to become so-called 'PAT' solutions: Process Analytical Technology (PAT) is the design and control of manufacturing processes through real-time measurements with the goal of ensuring final product quality.
A typical experimental apparatus for batch crystallization may utilize various *in situ* sensors to monitor the crystallization process. Among the instrumental techniques that have gained prominence so far are near infrared (NIR) and Raman spectroscopy for monitoring polymorphic forms, FT-IR spectroscopy for monitoring solution concentration and Focused Beam Reflectance Measurement (FBRM) and Process Video Microscopy for understanding particle size, particle shape, and particle population.
This application note describes the use of a new Slurry Flow Cell in research-scale experiments to prove the excellent suitability of the X-ray diffraction technique (XRD) to act as

Crystallization processes
In the pharmaceutical industry, a significant proportion of materials is produced in crystalline form. Many of these crystals are obtained by nucleation and growth from solution in industrial crystallization processes. Whether for formation or purification of the product, investigation of intermediates, or on the other hand prevention of crystallization in amorphous products, crystallization is always a key aspect of pharmaceutical manufacturing and development, with a significant impact on the efficiency and profitability of the overall process.

Cost for a single batch of active pharmaceutical ingredient could easily be $1 to $2 million. Undetected fluctuations in the crystallization process can alter the crystal structure, which can affect the safety and the bioavailability of the product. Failure to meet product specifications incurs significant costs, for example when the drug needs to be reprocessed or even destroyed. The ability to reliably monitor these precious crystallization processes on-line has become a strong need in the industry.

Summary
This application note describes the use of a PreFIX Slurry Flow Cell for research-scale crystallization experiments on a PANalytical X-ray diffractometer. Transmission experiments were performed to monitor and analyze crystallization processes from the very first stages up to high crystallite concentrations.
The crystallization of DL-alanine, an amino acid, was investigated under different pH conditions. DL-alanine is known to typically grow in a needle-like crystal morphology, with its main growth direction along the c axis. In our experiments at a pH of 6 and at a pH of 9.5, first crystallization is most pronounced in crystal directions characterized by the appearance of the (002)- and (311)-reflection. On the other hand, the crystallization process at pH 3.5 was much slower and the crystal morphology was less pronounced. The Slurry Flow Cell also proved to be suitable for small-angle X-ray scattering (SAXS) measurements.

on-line sensor. The Slurry Flow Cell is integrated in a XRD system and allows the real-time monitoring and analysis of solid phase growth and polymorphic phase transformation during the crystallization process. Since on-line analysis also enables the detection and analysis of intermediate products it is very useful for the understanding of the processes and process kinetics in order to get the final product. This process understanding is the most critical part for a QbD (Quality by Design) approval. Information about parameters that influence the crystallization process (solvents, concentrations, pH, stirrer geometry and speed, reactor geometry, temperature, temperature ramps, pressure, etc.) are of crucial importance here.

Even though this first version of the cell does not yet have an active temperature control, it covers main applications for the pharmaceutical research and scale-up.
The Slurry Flow Cell system was constructed with expert advice from Malvern Instruments (UK) and uses several proven elements from Malvern particle size instruments to prevent influencing particle morphology.

Applications for *in situ* studies

- Crystallization from supersaturated solutions: investigation of intermediates (polymorphs) and hemi-hydrates (which may differ from the final product) during the crystallization process
- Solvent / antisolvent reactions (e.g. in cleaning processes)
- Parameter variation in the crystallization process (pH, (anti-) solvent concentration, etc.)
- Small-angle X-ray scattering (SAXS) studies of early crystallization stages or nanoparticles
- Scale-up investigation

X-ray diffraction

X-ray diffraction is known to be the most suitable technique to analyze any crystallized (solid) compound, both qualitatively on its crystal forms and quantitatively on the concentrations of the various solid forms present.

More information on the X-ray diffraction technique and instrumentation can be found on our website:

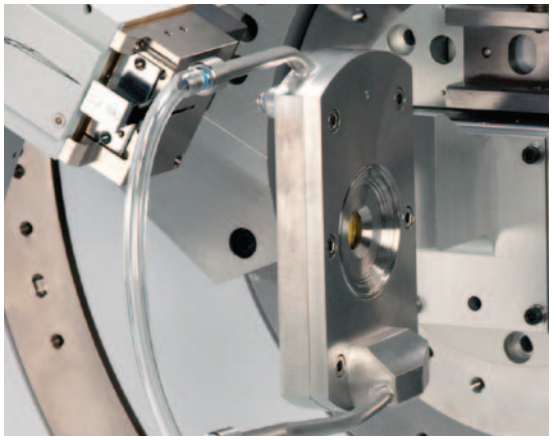
www.panalytical.com

Instrument setup

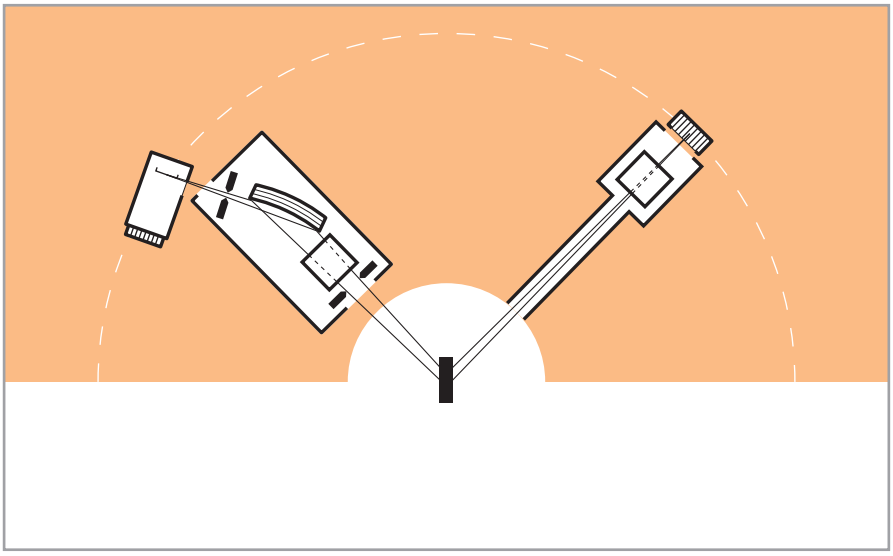
Instrument	Empyrean or X'Pert PRO MPD
Incident beam optics	Focusing mirror with incident beam anti-scatter slit, 0.04 rad Soller slits
Sample stage	PreFiX Slurry Flow Cell
Diffracted beam optics	Anti-scatter slits, 0.04 rad Soller slits
Detector	PIXcel^{3D} used in 1D mode, alternatively X'Celerator detector can be used
Scan parameters	13 – 32° 2θ, 0.013° step size
Scan time	Repetitive scans of 20 minutes

Diffraction system

For the crystallization studies we used a Slurry Flow Cell connected to a Malvern dispersion unit (Hydro 2000SM). The dispersion unit was set to 1500 – 2000 rpm to pump the slurry through the measurement flow cell. The cell was integrated on a PANalytical Theta-Theta diffractometer system, positioned in a transmission geometry mode. The system was configured with an incident beam focusing mirror, using Cu *Kα* radiation and the PIXcel detector on the diffracted beam side. Soller slits (0.04 rad) were used in both the incident and diffracted beam paths to limit the axial divergence of the beam.



PreFIX Slurry Flow Cell mounted on an Empyrean diffractometer



Beam path of the used configuration

Experimental results

Sensitivity determination
As a test to illustrate the sensitivity of the Slurry Flow Cell, small quantities of crystalline lactose were added to ethanol (0.5 and 1 wt %).
The 0.5 wt % lactose could easily be determined in this experiment after scanning for approx. 16 minutes in a 2theta range from 12 to 18 degrees (see Figure 1).

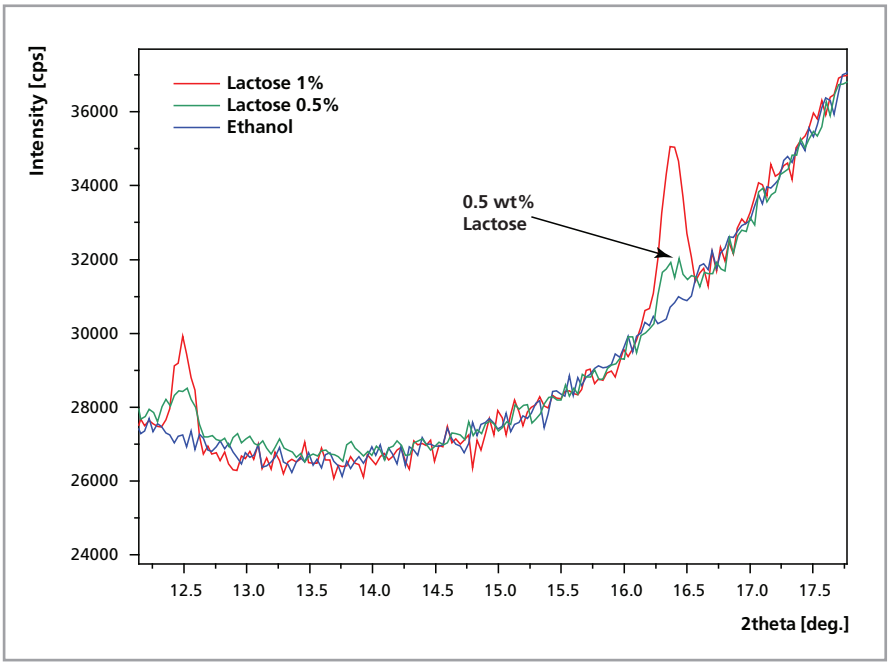


Figure 1. Sensitivity test of the Slurry Flow Cell. A quantity of 0.5 wt% lactose in ethanol (emulsion) can easily be detected

Crystallization by cooling a supersaturated solution
A small amount of DL-alanine, an amino acid with only one known (orthorhombic) crystal form, was dissolved in H_2O at 65°C to get a nearly saturated alanine solution (24.5 g DL-alanine per 100 ml water), which was allowed to cool slowly towards ambient condition, while maintaining a continuous flow through the Slurry Flow Cell, Figure 2. The crystallization process was monitored by making repetitive scans in the 2theta range 13-32 degrees (scan time 20 min).

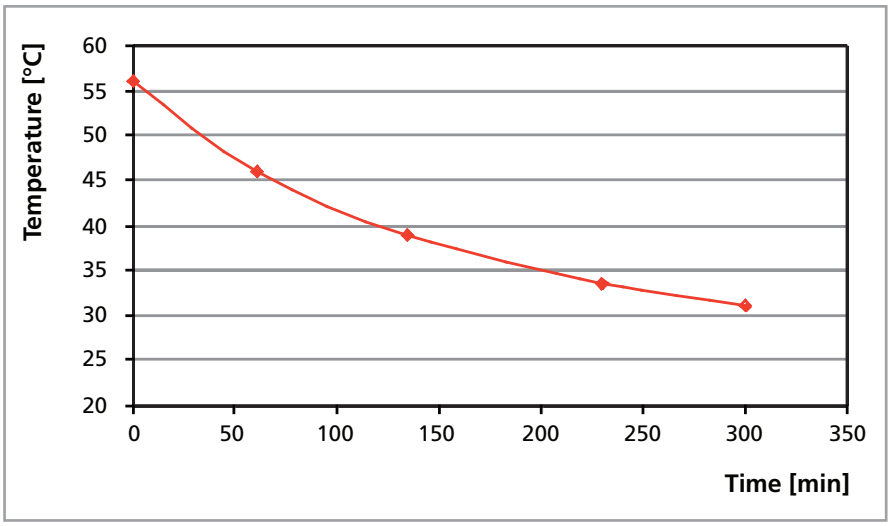


Figure 2. Temperature curve during cooling of the slurry

Amino acid molecules are so-called 'zwitterions', known to crystallize in aqueous solution when changing the pH of the nearly saturated solution.

The crystallization process was monitored in three solutions with different pH: the starting solution at a pH of 6.11 (the pH of saturated DL-alanine solution), one solution with a pH of approximately 9.5 (addition of NaOH) and one with a pH of approximately 3.5 (addition of HCl).

The different crystallization conditions show distinct differences in crystallization initiation and crystal morphology.

Crystallization in the pH 6 solution starts after more than 10 h - (Figure 3), whereas at a pH of 9.5 first crystals are formed much faster (only 4 h) (Figure 4). At a pH of 3.5 the crystallization process is very slow. Crystallites are observed after more than 30 h - (Figure 5). DL-alanine is known to grow in a needle-like crystal morphology, with its main growth direction along the c axis. In our experiments at a pH of 6.11 and at a pH of 9.5, first crystallization is most pronounced in crystal directions characterized by the appearance of the (002)- and (311)-reflection, only much later followed by fast increase of the (210)-reflection. Peaks become sharper and higher in intensity in the course of the crystallization, indicating increasingly larger crystals (Figure 6). In the very slow crystallizing condition at a pH of 3.5, the crystal morphology is less pronounced, while the broad peaks point towards smaller crystallites (Figure 5).

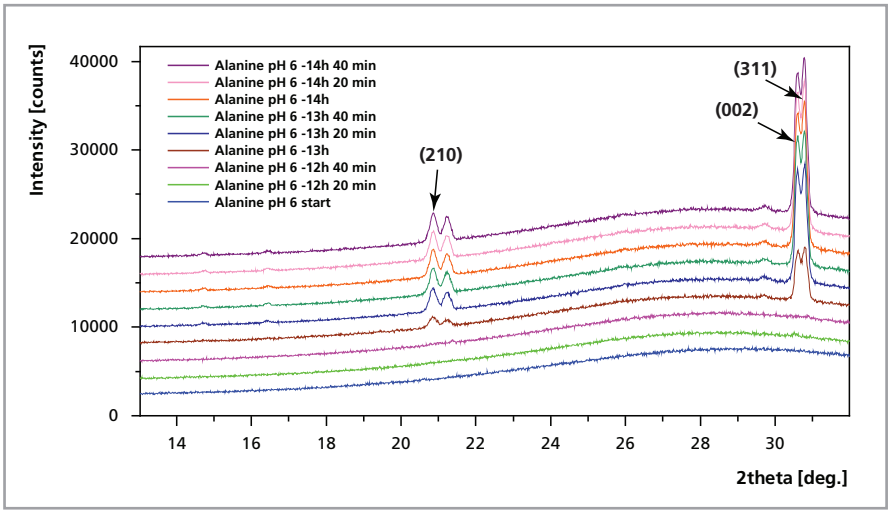


Figure 3. DL-alanine crystallization at a pH of 6. The crystallization is starting about 13 hours after initiation of the cooling process, indicated by the appearance of the (311) and (002) reflections.

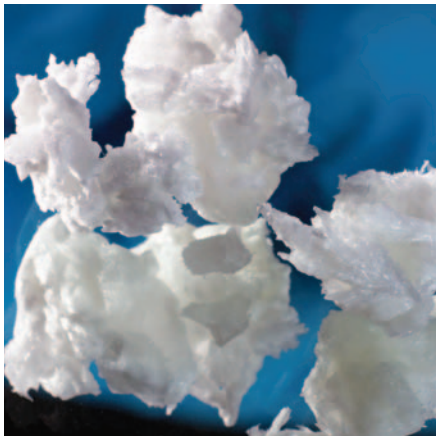
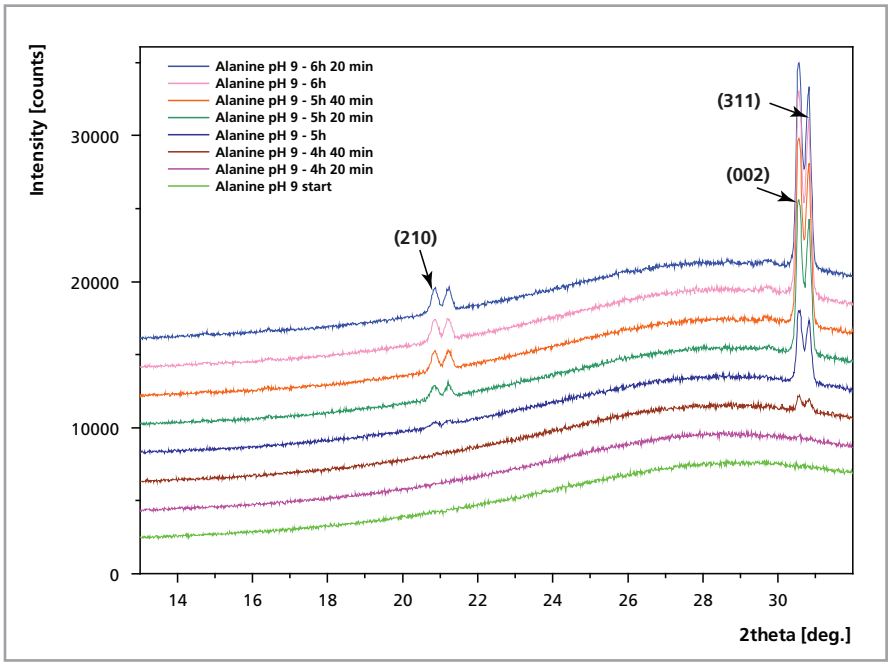
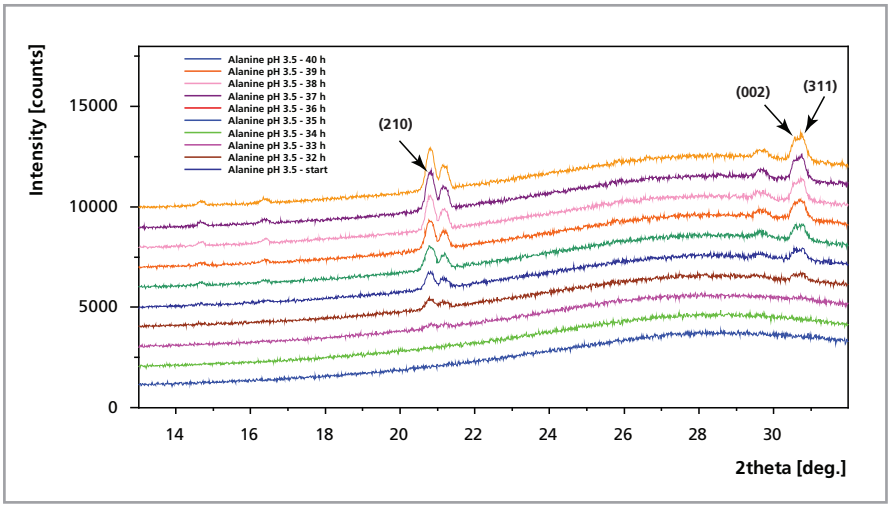


Figure 4. DL-alanine crystallization at a pH of 9.5: crystallization occurs after 4.5 hrs



Experimental results (continued)



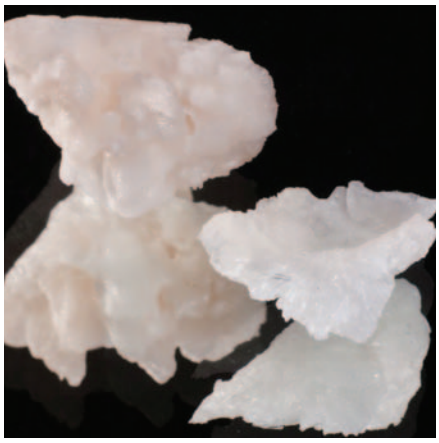
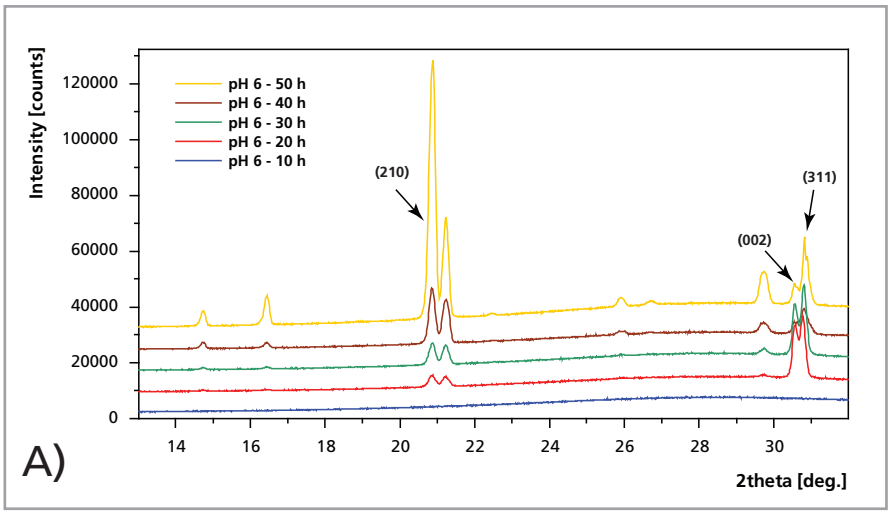


Figure 5. DL-alanine crystallization at a pH of 3.5: crystallization occurs after approx 33 hours with only very slow growth



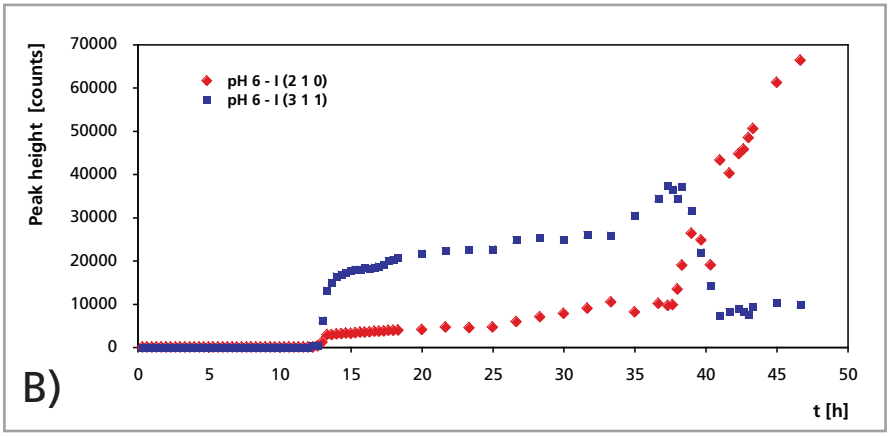


Figure 6. DL-alanine crystallization at a pH of 6. After a starting crystal growth with pronounced peaks at the (311) and (002) reflections, the (210) reflection starts to grow stronger after 38 hours.
A) Diffraction patterns during the crystallization process at a pH of 6.
B) Evaluation of peak heights of the (311) and (210) reflections during the crystallization process.

SAXS measurements

Small-angle X-ray scattering (SAXS) allows to determine particle size distributions of nanoparticles in powder materials, nanocomposites or dispersions. Figure 7 shows SAXS measurements on low concentrations of silica nano-particles with a radius of R_{50} = 15 nm (Ludox® TM-50 Silica) dispersed in water. For these measurements the incident beam optics have been adapted with SAXS slits. The particles have been measured in the flow cell with different concentrations. A concentration of 0.1 % silica particles in water could still easily be detected. The evaluation of the measured particle sizes with the PANalytical software package EasySAXS confirms the particle radius of 15 nm for all concentrations measured.

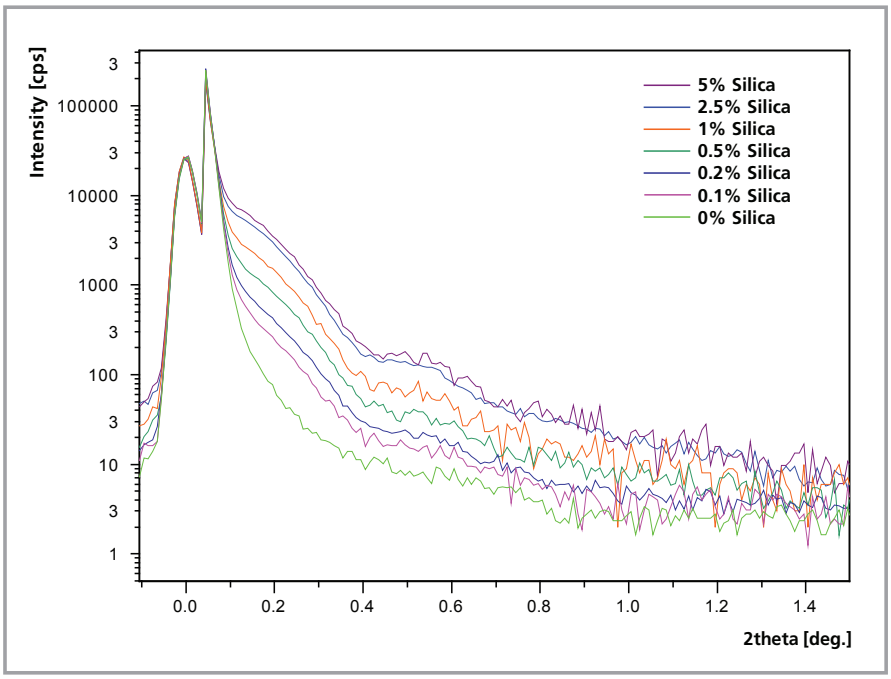


Figure 7. Normalized SAXS measurement on Ludox® TM-50 Silica nano particles with a radius of 15 nm in the flow cell. The particles were dispersed in water and were measured floating through the cell.

Conclusion
We have shown that the demonstrated experimental setup can be used to monitor and analyze crystallization processes from the very first stages up to high crystallite concentrations. XRD proved to be an ideal QbD technique for the investigation of the parameters influencing the different stages of the crystallization.

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Global and near

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PANalytical is the world's leading supplier of analytical instrumentation and software for X-ray diffraction (XRD) and X-ray fluorescence spectrometry (XRF), with more than half a century of experience. The materials characterization equipment is used for scientific research and development, for industrial process control applications and for semiconductor metrology.
PANalytical, founded in 1948 as part of Philips, employs around 1000 people worldwide. Its headquarters are in Almelo, the Netherlands. Fully equipped application laboratories are established in Japan, China, the USA, and the Netherlands. PANalytical's research activities are based in Almelo (NL) and on the campus of the University of Sussex in Brighton (UK). Supply and competence centers are located on two sites in the Netherlands: Almelo (development and production of X-ray instruments) and Eindhoven (development and production of X-ray tubes). A sales and service network in more than 60 countries ensures unrivalled levels of customer support.
The company is certified in accordance with ISO9001-2008 and ISO 14001.

The product portfolio includes a broad range of XRD and XRF systems and software widely used for the analysis and materials characterization of products such as cement, metals and steel, nanomaterials, plastics, polymers and petrochemicals, industrial minerals, glass, catalysts, semiconductors, thin films and advanced materials, pharmaceutical solids, recycled materials and environmental samples.

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