BRAVE USE CASES & TESTIMONIALS

BRAVE Analytics' devices in action

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ANALYTICS

Research on drug targeting of the early processes of biomolecular condensate formation

Understanding dynamic liquid-liquid phase separation (LLPS) processes by observing the formation and size distribution of the proteins as they change over time.

Challenge

Distortions of biomolecular condensates are hallmarks of a plethora of age-related diseases, such as cancers and neurodegenerative diseases. Prof. Tobias Madl investigates the (dys)regulation and drug targeting of the early processes of condensate formation, when condensates are small. He used BRAVE B-Curious to study the size and dynamics of biomolecular condensates.

His challenges were:

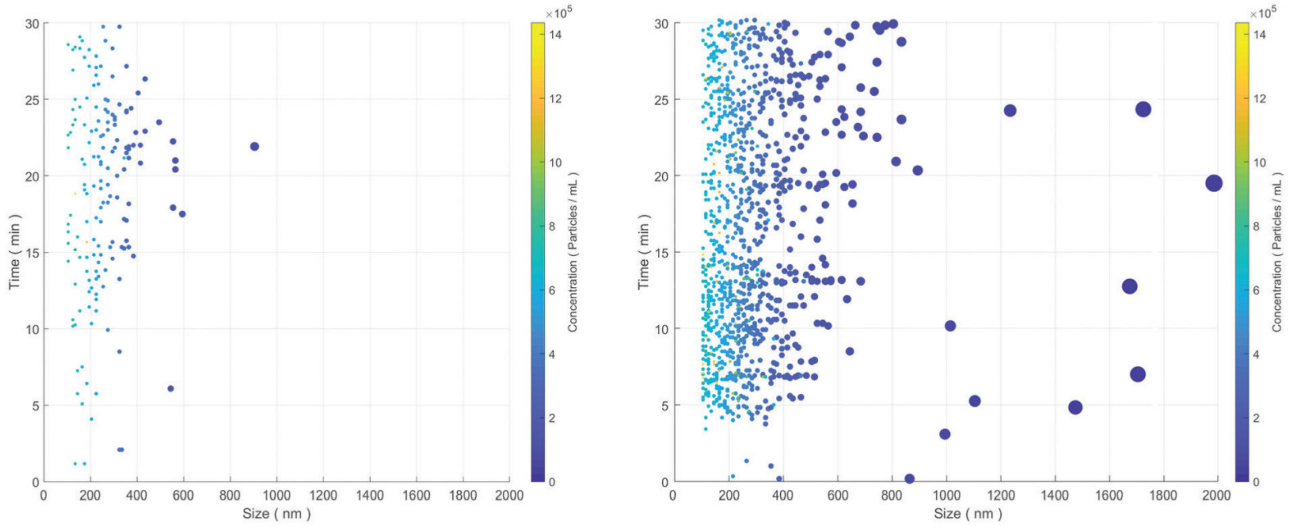
- Other methods did not allow live/time-resolved measurements to monitor changes of molecular condensates and PSD as they happen.
- Only small sample amounts are available (patient samples); high protein and RNA concentrations are required to obtain results.
- Particle size is very small; size distribution range is wide.
- Microscopy does not resolve the formation processes in the early stages when condensates start to form and proteins start to interact with RNA.

Application highlights

Prof. Madl carried out time-resolved measurements on different concentrations of proteins and RNA. The measuring results clearly showed the kinetics of LLPS processes and allowed comparison of the formation of particles in the presence of different RNA concentrations.

Benefits of OF2i:

- Visualizing the formation and size distribution of proteins as they change over time in one seamless and complete measurement.
- Results with single-particle sensitivity, even for condensate particles in the size range 10 nm to 2000 nm.
- Measurements on low-volume samples of 80 µL to 100 µL and at concentrations relevant for drug discovery.



Time-resolved PSD: Particle formation processes during liquid-liquid phase separation (LLPS) with low (left) and high (right) RNA concentrations over 30 minutes. © BRAVE Analytics & Marko Šimić

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Continuous online monitoring of high-pressure homogenization processes

Online monitoring of the particle size distribution (PSD) of emulsions for intravenous application of pharmaceuticals, parenteral nutrition during homogenization to ensure formulation stability.

Challenge

The particle size distribution of parenteral emulsions is a critical quality attribute (CQA).
Christoph Koeth and his colleagues tested a number of sensor systems for online monitoring of their emulsions. A chance meeting with Chris Hill and Gerhard Prossliner from BRAVE Analytics led to the joint collaboration and development of the BRAVE B-Continuous online sensor.

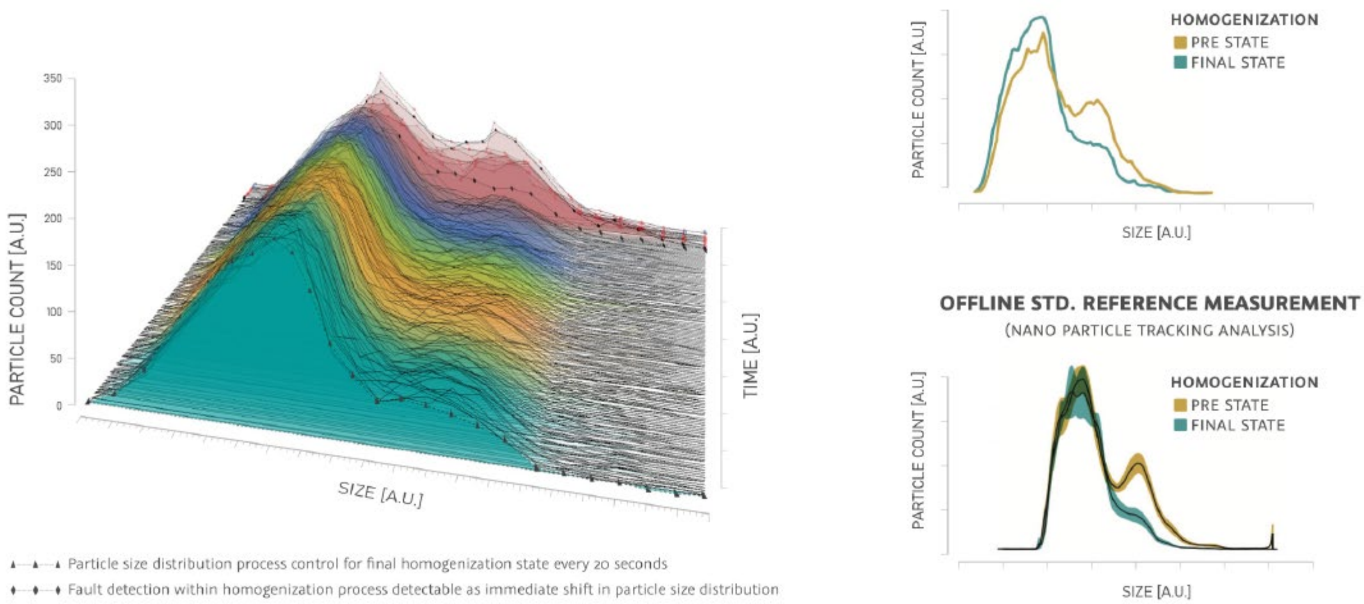
Application highlights

The BRAVE B-Continuous online unit (including a continuous and automated online sample preparation unit) was installed in a four-stage homogenization pilot plant. After the first step, the particle size is reduced from 3 µm to approx. 0.3 µm. The process analyzer continuously monitors the PSD distribution and automatically evaluates data such as D-values to assess the efficiency of the first homogenization step.

The challenges are:

- The time lag inherent with laboratory measurements leads to inefficient production processes.
- Offline measurements are time-consuming and require qualified personnel.
- Current offline methods provide results too late for process optimization.
- Current measurements do not capture all fluctuations and anomalies.

- The values are sent to the LIMS every 20 seconds.
- A better understanding of the homogenization process leads to more efficient production and huge savings in product waste.



OF2i® time-resolved online characterization as number-based size distributions versus nanoparticle tracking analysis of different processing steps in looped emulsion production. Timestep 10: fault detected within homogenization. © BRAVE Analytics & Marko Šimić

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Calculating the filtration efficiency of drinking water filters at high throughputs

Fähzan Ahmad (founder and CEO of Klar2o) needed a precise and continuous input/output analysis for an innovative drinking water filter.

Challenge

Klar2o needs to verify the filtration efficiency of their filters. The concentrations are very low.

In a feasability study, we used the BRAVE B-Curious nanoparticle analyzer to measure polystyrene beads (300 nm) before and after filtration with the Klar2o system.

Experiment

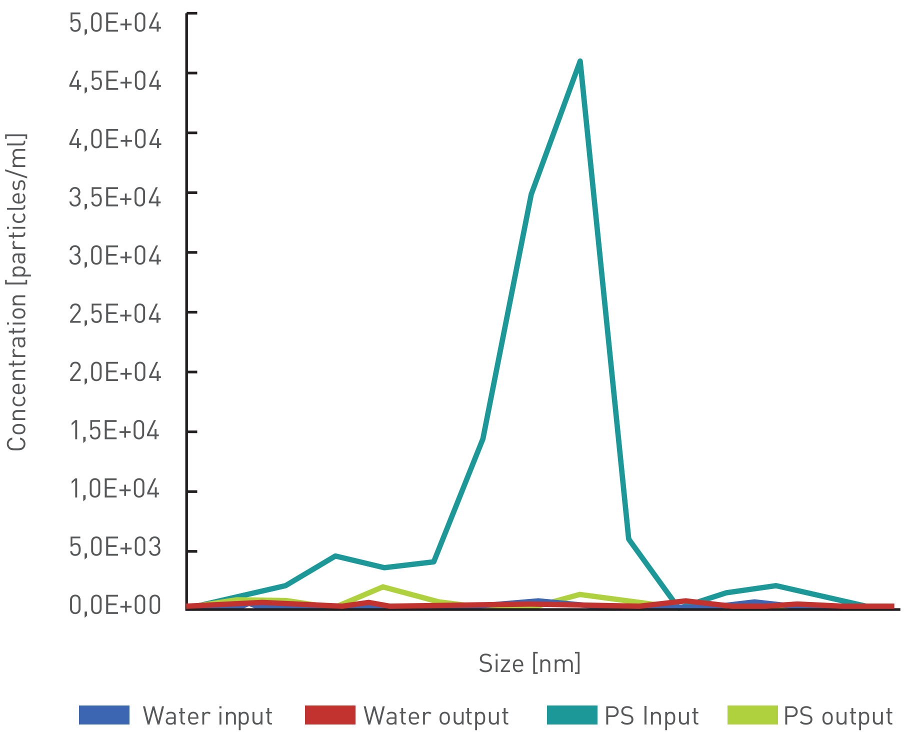
The input sample was water with 1.22 E05 PS (polystyrene) beads per ml, measured with OF2i® without sample dilution.

The sample was filtered with a Klar2o filter as per the manufacturer's instructions and measured with OF2i® again.

The number of polystyrene beads detected in the output sample was 7.25 E03 particles per ml. The sizing range was 215 nm to 355 nm.

Application highlights

- Continuous measurement at high sample throughput
- Single-particle precision: OF2i® detects down to a few particles per milliliter
- No sample preparation
- Results in 2 to 3 minutes In this measurement, 98.8 % of particles sized 215 nm to 355 nm were removed by the filter.



Water sample measured before and after filtration with Klar2o system, showing the particle concentration of the water (input, output) and the concentration of polystyrene beads (input, output)

Determining the particle concentration of PIT nanoemulsions

In a feasibility study with Henkel, we tested two innovative emulsion types to better understand their composition. The results revealed significant differences between the samples.

Challenge

Phase inversion is an important industrial process to make stable emulsions e.g. for personal care products. After phase inversion, the liquid-in-liquid dispersions have a droplet size in the nano-range. This brings a number of benefits due to e.g. high surface area per volume, high stability and other desirable properties. However, it is not always easy to control and predict the final droplet size during the production process.

Application highlights

During measurement the live datastream of particles already gave an indication of differences between the two samples. The number of particles (shown as strips of light) passing through the measuring cell was noticeably lower for Emulsion 1 than for Emulsion 2 (see Figure 1).

Results

Subsequent comparison of the OF2i® data for both samples revealed that the object concentration (number of objects) of Emulsion 2 was 4.8 times higher than for Emulsion 1 (see Figure 2).

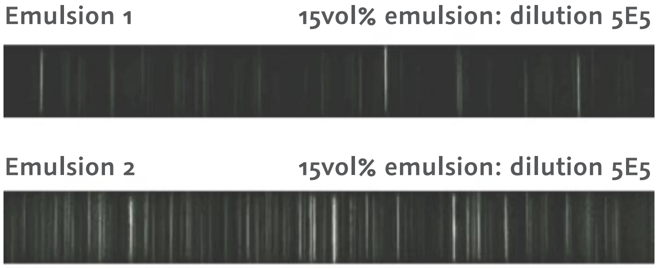


Figure 1: Comparison of the live datastreams of Emulsion 1 (left) and Emulsion 2 (right) showing noticeably more particles passing through the cell during measurement of Emulsion 2

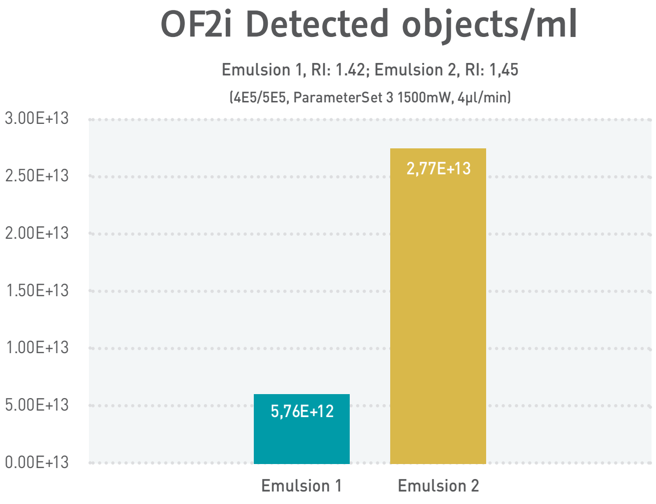


Figure 2: Results of the OF2i® measurement of object concentration on Emulsion 1 and Emulsion 2

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Monitoring the leaching of micro- and nanoplastic particles from bottles into ultrapure water

Continuously measuring plastic particle contamination and the leaching processes of different polypropylene bottles and comparing them to glass bottles.

Challenge

Katharina Heider (a researcher at a leading Austrian university) was unable to obtain insights into leaching from polypropylene bottles until she performed OF2i® measurements.

Previously there was no technology available to measure concentrations down to a few particles per milliliter.

Experiment

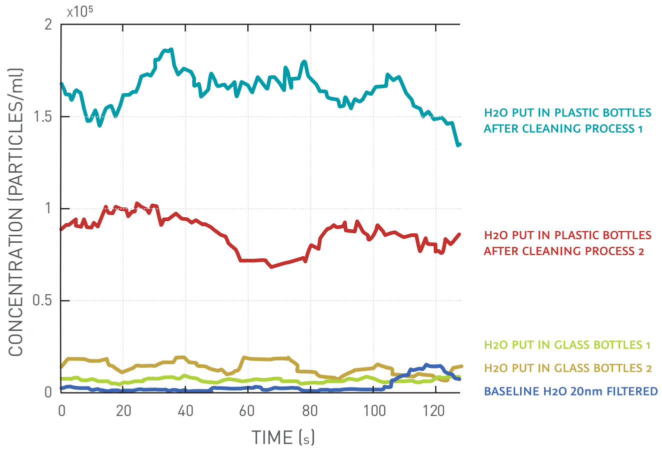
In the experiment, Katharina sterilized the bottles according to the manufacturer's recommendations and performed various cleaning procedures using 20-nm-microfiltrated ultrapure water. No further sample preparation was required.

- After taking the baseline concentration Katharina measured the particle concentration in glass and plastic bottles and repeated the cleaning and measuring procedure three times.
- They discovered that different contaminations are seen for different cleaning procedures.
- For the experiment, a relatively large sample had to be pumped through the analyzer over a long period of time, in this case 50 minutes.

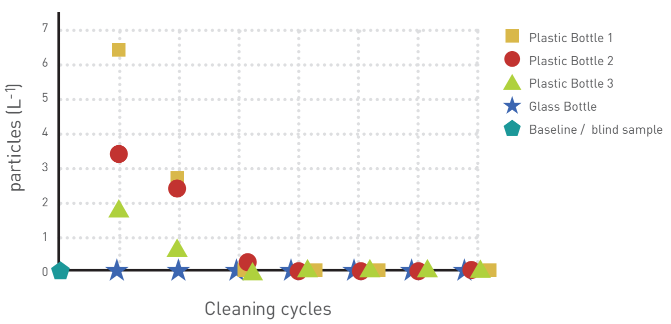
One of the takeaways from the experiments is that particles are leaching into the liquid as soon as the liquid is in the plastic bottle.

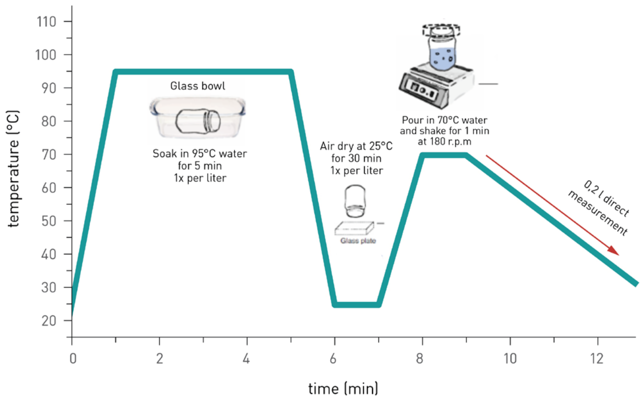
The challenges were:

- No possibility for live or time-resolved measurements to monitor particle concentrations.
- The particle concentrations are very low and therefore a very large sample is required.
- The results of such low concentrations are not statistically relevant and almost impossible to measure with other methods.



Time-resolved particle concentrations measured in plastic and glass bottles with baseline © BRAVE Analytics





Particles detected in plastic and glass bottles after cleaning cycles with baseline (left); steps of the experiment (right) © BRAVE Analytics

BRAVE ANALYTICS

High-throughput detection of nanopollutants

Gregor Marolt (assistant professor at the University of Ljubljana) was looking to detect and measure the particle size of low concentrations of nanoparticles in surface water.

Challenge

The wear on tires results in over 1000 tons of nanoplastics landing in soils, rivers and seas each year. To enable low-budget detection of car tire nanopollutants in surface water, Assistant Professor Gregor Marolt and his research group at University of Ljubljana are developing an innovative screen-printed sensor technology. He needed a quick and reliable way to verify his measurements.

Application highlights

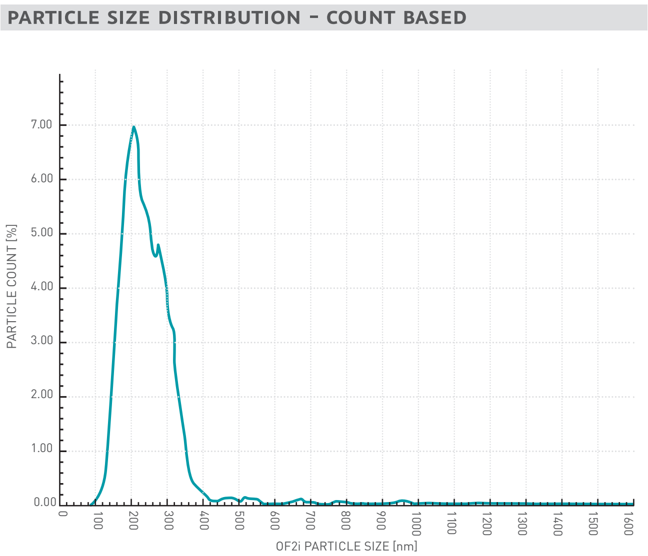
- Quantifying the differences between differently filtrated samples and measure D-Values, concentration and particle size
- High-throughput measurement with results ready after 5 minutes
- Detecting nanoplastics in real-time at ultra-low concentrations (down to a few particles per mL)

Experiment

The goal was to quantify the differences between differently filtrated samples. Comparing the number-based distributions and corresponding parameters (D90 and D3/4) for these two samples show, as expected, that for the suspension filtered with 0.4 µm more of the bigger particle fractions are present in the sample.
During the measurement over 300 particles were measured with single-particle sensitivity, also capturing unknown bigger particles (probably agglomerates).

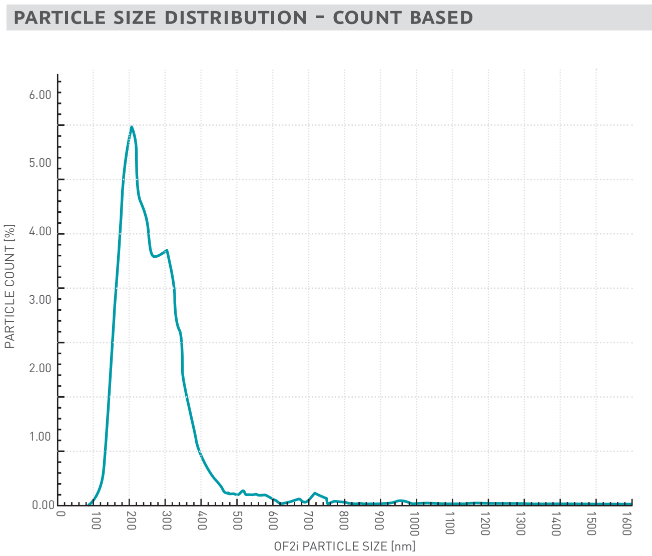
In the future, the system will be used for online real-time monitoring during development of the sensing technology. The inlet and outlet of the sensor will be connected to a small volume of analyte and concentration / PSD will be monitored dynamically as the particles are expected to collect on the electrode.
With the information it will be possible to correlate real-time data obtained with OF2i with the change of signal measured by the sensing technology of the group.

Sample filtered with 0.22 µm filter



Mean concentration ~8E5 objects
D90 - 321.2nm (90% of the population is smaller than 321 nm)

Sample filtered with 0.45 µm filter



Mean concentration ~E6 objects
D90 - 406.7 nm (90% of the population is smaller than 406.7 nm)

Comparison of number-based distributions for two samples (left: suspension filtered with 0.22 µm filter; right: suspension filtered with 0.45 µm filter)

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Testimonials

Assistant Prof. Gregor Marolt
Faculty of Chemistry and Chemical Technology, University Ljubljana

Detecting nanopollutants in surface water

"We see great potential in using OF2i® to support us with verification and development of our sensing technology. BRAVE Analytics is an outstanding innovation partner in solving one of the most pressing challenges of modern society – enabling better monitoring of ground and drinking water quality."

Univ. Prof. Dr. Madl
Medical University Graz & Gottfried Schatz Research Center

Understanding dynamic liquid-liquid phase separation (LLPS) processes

"As the small condensates we are interested in cannot be detected with microscopy their study remains a blind spot. The OF2i® technology promises to close this gap and to provide unprecedented insights into the processes driving early condensate formation and targeting of condensates."

Christoph Koeth
Senior Director Innovation, Fresenius Kabi, Austria

Online monitoring of total parenteral nutrition products produced in high-pressure homogenization processes

"With OF2i® we can now go way beyond our previous capabilities and see great potential to replace several devices. We are investing in a future which will provide us with real-time information about the product quality and may deliver insights beyond currently established controls."

Lars Vierkotten
Manager Scalp Tissue Engineering, Henkel R&D, Germany

Understanding phase inversion in emulsions

"We see great potential for OF2i in the online measurement of particle concentrations enabling characterization and further development of our formulas on the road to innovative cosmetic products."

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Testimonials

Harald Fitzek
Institute of Electron Microscopy and Nanoanalysis, TU Graz, Austria

Developing new methods for particle analysis

"The addition of a Raman spectrometer to the OF2i device makes it possible to not only count but also identify individual microparticles in liquids. The key strengths of this combination are that it requires little sample preparation and a large number of particles can be measured."

Kathrin Heider
Montan University, Leoben

Monitoring the leaching of micro-and nanoplastic from bottles into pure water

"The OF2i® method enables an in-depth and seamless observation of smallest amounts of plastic particles leaching into very large samples of water."

Sebastian Schwaminger
Ass.-Prof. Medical University of Graz

Investigating drug delivery formulations

"The OF2i® technology is an excellent tool for investigating the time-resolved degradation of drug delivery systems."

Dr. Allan Philippe
Group for Environmental and Soil Chemistry at the RPTU Kaiserlautern-Landau, Germany

Researching in the field of nanomineralogy

"Great tool for nanomineralogy: measurements with the OF2i®-Raman device shed light on the intricate composition of soil samples. There is potential to deliver Raman spectra for hundreds to thousands of soil particles per sample."

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Testimonials

Dr. Alexandros Zoikos Karathanasis
CEO of Creative Nano

Monitoring electroplating processes

"Having the OF2i® test installation helps us understand our electroplating processes and deliver the best possible product."

Dr. Catarina Coelho
R&D department, Fluidinova, Portugal

Monitoring reactions during production of nano-hydroxyapatite for oral care products

"The BRAVE online device will help us monitor critical quality attributes during our synthesis reactions."

Martin Miltner
CEO of Lignovations

Monitoring particle size in the production of lignin-based sun screen

"We are in need of a reliable at-line particle size measurement system that can be operated in a production environment and delivers particle size measurements at different locations along the production line."

AREAS OF EXPERTISE

BRAVE B-Continuous is currently in use at a pilot plant producing pharmaceuticals. BRAVE B-Curious and BRAVE B-Elementary are at work in research and industry.

REAL-TIME PROCESS CONTROL

and automated QC in liquid (nano)particle production (e.g. emulsions, LNPs)

QUANTIFICATION & ANALYSIS

of nano- and micropollutants (e.g. degradation processes), water for injections, microplastics in fluids

AGGREGATION DETECTION

for R&D and production and for QC (e.g. monoclonal antibodies, proteins)

ENABLING NEW INSIGHTS & DISCOVERIES

and satisfying intrinsic curiosity in basic research and R&D (formation of e.g. LLPS, degradation processes of e.g. microplastics)

For more information and updated use cases, see https://braveanalytics.eu

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