Analysis of particulate substances in injectable medication – All roads lead to µ-FT-IR imaging

Application Note MIC422

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Why care about particles?

Detecting and identifying micro-particles are essential for many applications, as particles can be the source of contamination in many products, e.g. cleaning agents, polymers, industrial oils, in health and beauty products, and in pharmaceuticals.

Considering the ubiquitous presence of particles in industrial production, particle analysis becomes a mammoth task for analytical labs. This especially goes for particulate substances in injectable medication, where contaminations must be avoided at all costs.

Here, complications caused by particulate matter can be quite severe. Intravascular particulates can trigger a range of biological responses such as blockage, thrombosis, or even organ damage. Extravascular particulates can trigger an immune response and cause inflammation, or even tissue damage.

Naturally, pharmacopeias are interested in tracing the origin of the particles to prevent recurrence. Usually, the source of particle contamination is either the container or the production process itself. In some cases, however, the degradation of pharmaceutical products, e.g., protein aggregation, can also be the source of undissolved solid particles.

What does regulation say about particles?

Testing pharmaceutical solutions for particulate matter is regulated by Ph. Eur. 2.9.19 and Ph. Eur. 2.9.37, USP <766>, USP <787> and USP <788>, and JP 3.04 and 6.03, as well as the ChP 0903, and ChP 0904. The USP <766> regulates the detection and identification of particles larger than 1 µm, by optical microscopy, while USP <787> is an alternative to USP general chapter USP <788> for Particulate Matter in Injections.

USP chapter <787> Subvisible Particulate Matter in Therapeutic Protein Injections was evolved to address the limitations of USP for therapeutic proteins by providing a smaller-volume testing framework to address the effects of sub-10 µm proteinaceous particles. According to the USP, visible particles have a diameter ≥ 50 µm, and sub-visible particles are smaller than 50 µm.

For Small Volume Injectables (SVI ≤100 ml), a maximum of 6000 particulates larger than 10 µm and 600 particulates larger than 25 µm are allowed per container. The number of particulates in Large Volume Injectables (LVI > 100 ml) should not exceed 25 particles larger than 10 µm per ml, and 3 particles bigger than 25 µm per ml.

How do you detect such particles?

When you want to detect and identify particulate matter, optical microscopy scores with high spatial resolving power, but fails to resolve the chemical composition. In contrast, FT-IR microscopy not only provides high resolution but also comprehensive chemical information that can be used to identify found particles.

It thereby reveals the chemical composition of spatially defined sections of a sample. Furthermore, the USPs <788> and <787> recognize FT-IR microspectroscopy as a suitable tool for the characterization of subvisible particulate matter in therapeutic protein injections.

This application note presents two examples of using FT-IR microscopy and imaging for the identification of particles in liquid formulations.

Application example #1: FT-IR microscopy approach; Antibody solution contaminated with particulates

Antibodies have numerous applications in the pharmaceutical industry and naturally, impurities and contaminations reduce the quality and specificity of antibody solutions. Therefore, we analyzed an antibody solution for eventual traces of particulates.

First, to separate particulate matter from the liquid phase, the antibody solution was filtered by utilizing a 25 mm IR transparent Anodisc (Al_2O_3) filter with 0.2 µm pore size. Afterward, an overview image of the filter was automatically acquired with the high-res visual camera of the LUMOS II.

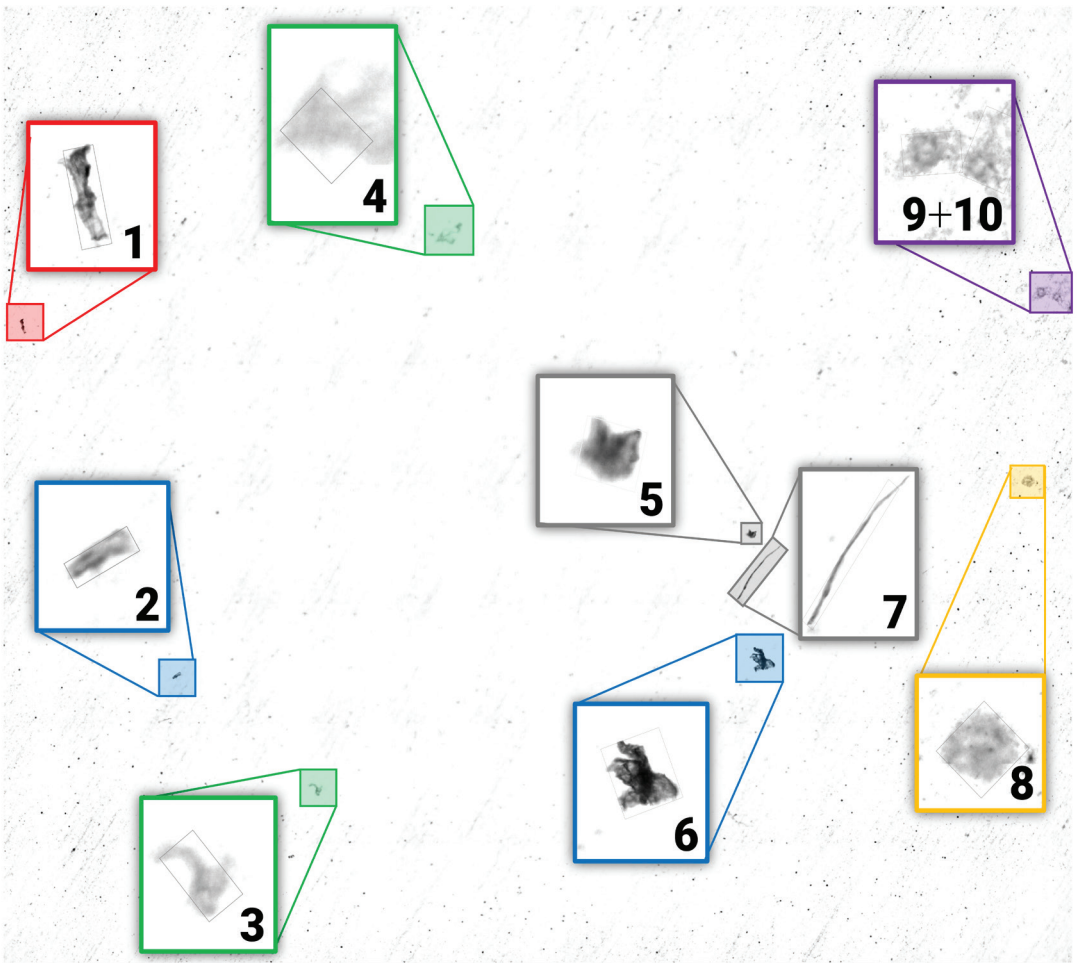

Fig. 1
Visual image of particles on an Anodisc filter. Ten particulates were detected and superimposed as enlarged images. The knife-edge aperture frame (gray rectangles) is set automatically for each particle.

Based on their visual contrast, particles were detected using the Bruker Particle Finder routine, revealing the presence of ten particles. A measurement point was automatically assigned to each found particle.

The detected particulates are numbered, enlarged and superimposed onto the visual overview image (Fig. 1). The magnified view also shows the size of the knife-edge aperture frame (gray rectangles) which is automatically matched to the shape of each particle. By doing so, the LUMOS II achieves highest spectral quality.

In a fully automated workflow, a µ-FT-IR spectrum was then collected at each particle´s location by a transmission measurement. Every single particulate is identified successfully by the OPUS Cluster ID based on its FT-IR spectrum (Table 1).

Table 1
Identity of the detected particulates (Fig. 1) revealed by a database search of the corresponding FT-IR spectra.

ID	Substance
1	PF (Phenol formaldehyde resins)
2	Cellulose
3 & 4	PARA (Polyaryl Amide)
5	PS (Polystyrene)
6	Cellulose
7	PS (Polystyrene)
8	PA12 (Polyamide)
9 & 10	Polyamide / Polyether Block Amide

In conclusion, utilizing transmission µ-FT-IR-analysis by a single-element infrared detector and the Anodisc filter leads to the successful detection and identification of particulates.

Application example #2: FPA imaging approach; Antibody solution contaminated with particulates

For this example, the sample remains unchanged. However, instead of the conventional FT-IR µ-spectroscopy approach, we utilize the FPA detector of the LUMOS II to create a chemical image.

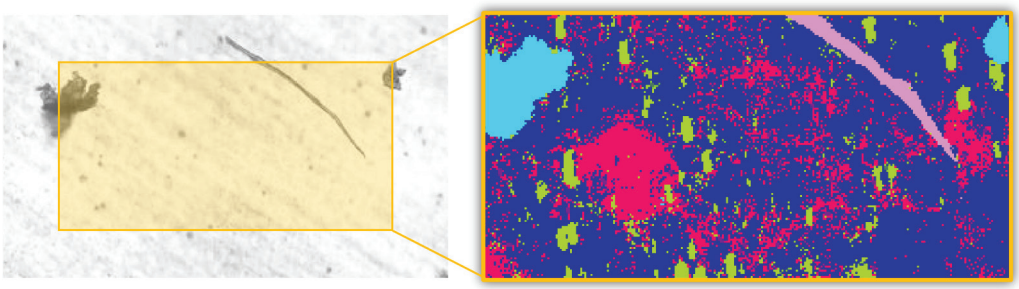

Fig. 2
Visible image of the filter and the particulates (left). FT-IR imaging of the area of interest was performed by the FPA detector (right). The IR chemical image identifies several chemical components by their spectral signature, including cellulose (pink) and polystyrene (blue). Silicone oil (red), and protein particulates (green) were invisible to optical microscopy but were revealed and identified.

The visible overview image of the filter and the particles was recorded for reference and a chemical image was acquired by the LUMOS II utilizing its focal plane array detector in transmission (Fig. 2). Since FPA detectors facilitate IR imaging at the highest speeds, the entire region of interest was measured in less than a minute and with a spatial resolution of 5 µm/pixel.

The dataset was processed completely autonomously using Bruker's Adaptive Chemical Imaging AI algorithms and several clusters of particulates were identified (Fig. 2). The fiber visible to optical microscopy was identified as cellulose (pink), and two other visible particles were found to be polystyrene particulates (turquoise).

However, the FT-IR imaging approach reveals **much more**. In particular, silicone oil (red) and protein clusters (green) that were initially invisible were successfully detected and identified, as shown in Figure 2. Both the detection and the identification of the particulates was solely based on the collected FT-IR signature of the particles. This means that no particle remains undetected.

Conclusion

Infrared chemical imaging by utilizing the LUMOS II FPA detector is by far superior to other commercially available µ-FT-IR methods for microparticle analysis. It delivers a perfect combination of high-speed and high-resolution chemical imaging.

Here the chemical composition of the sample is deciphered independent of the contrast of the visible image. This is a major advantage of FT-IR imaging compared to optical microscopy. For the investigation of particulates in injectable solution and microparticle analysis in general, we strongly recommend FPA chemical imaging as the superior method.

Instrumentation

The Bruker FT-IR imaging microscope LUMOS II (Fig. 3) is a benchtop solution with an integrated FT-IR spectrometer. It offers more space for sample preparation, more speed for chemical imaging, and maximum performance in ATR, transmission, and reflection microscopy.

The standard TE-MCT detector and the compact, tightly sealed optical design enable the use of the system even in laboratories without access to liquid nitrogen and dry purge air. Furthermore, guided analysis workflows and autonomous AI-based data evaluation result in the highest productivity in your multi-user laboratory.

FPA imaging takes the speed and analytical quality of particle analysis to the next level. Thanks to automated instrument qualification routines according to all intl. pharmacopeia and full compliance with 21CFRpart11 the system is prepared for comprehensive validation.

Fig. 3
LUMOS II stand-alone FT-IR imaging microscope

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