Microanalytical Techniques for Identifying Nonprotein Contaminants in Biologics

by Mary Stellmack

Proteins can aggregate at any point during pharmaceutical manufacturing. Regulatory agencies pay special attention to aggregates that can enhance immune responses and cause adverse clinical effects and those that can compromise the safety and efficacy of a drug product. Biopharmaceutical companies have stringent quality control (QC) procedures in place to ensure that their final products are free of contaminants and defects, including protein aggregates. Trained QC inspectors, however, can typically see product defects or particulate material only as small as 50 µm by visual inspection. If contaminants can be identified at this stage, then steps can immediately be taken to correct problems in the manufacturing process. Even when particles can be seen, visual inspection alone is not usually sufficient to identify them and determine their source.

Isolating and analyzing small particles may require specialized technical skills and analytical instrumentation that an in-house QC laboratory does not possess. Many biopharmaceutical companies look to independent analytical or microanalysis laboratories that have the experience, skill, and necessary instrumentation to identify contamination and its source(s).

Although most contaminants consist of protein-based particulates, sometimes they consist of foreign materials (e.g., metal wear particles from production machinery, fibers from cleanroom wipes, glass particles, or even hair). Several current techniques and analytical methods are available to detect, isolate, and identify such impurities, whether they are protein-based or come from other sources.

Under the Microscope

Ideally, all sample manipulations take place in a cleanroom to eliminate the chance for cross-contamination. Upon sample receipt, microanalytical laboratories typically first examine vials of the protein solution by the naked eye, then using a stereomicroscope. An analyst views the solution through the vial walls using several specialized lighting techniques. Certain types of particles can be seen at this stage only with high-intensity fiberoptic lights.

Amorphous, stringy particles present in a solution suggest protein-based precipitation. Other particles — e.g., fibers, hair, or rubber particles — often can be seen at this stage too. The analyst then transfers the vial contents directly onto a filter in a vacuum-filtration apparatus. The most useful filters for this application are polycarbonate membranes with 0.2-µm or 0.4-µm pore sizes. The smooth, shiny surface of such filters allows an analyst to see small amounts of residue or particles on them and then easily remove those residues/particles for further analysis without any attached filter substrate.

On such a filter surface, protein particles typically form a continuous, amorphous, colorless residue that can be seen only using coaxial (reflected) light. Photo 1 shows proteinaceous residues on a filter as seen in two different kinds of light. Two other scenarios may occur: Proteinaceous residue may contain additional nonprotein foreign particles, or nonprotein foreign particles may be present without any amorphous proteinaceous residue (Photo 2).

Both scenarios point to a nonprotein contaminant having entered a drug solution. Scientists can use lighting techniques such as low-angle oblique lighting to discern foreign particles from a thick and gooey protein mass. Particles or amorphous residues can be removed from the filter with a fine tungsten needle and transferred to a glass slide for further examination or analysis. However, it is critical that particles/residues be removed while the filter is still somewhat wet because on drying, proteinaceous residue forms a brittle, insoluble film that is difficult to remove from a filter. It can form a solid crust over some foreign particles, as well.

To prevent premature drying, a filter can be placed in a Petri slide with a small drop of particle-free water under it, then covered. This technique keeps a filter wet for at least 30 minutes, which is long enough to remove particles/residues of interest. Foreign particles that are heavily coated with proteinaceous residue sometimes can be placed in a drop of particle-free water on a glass slide so that the gooey residue can be teased away from them with a tungsten needle.

Photo 1: Proteinaceous residue on a filter as seen in two different kinds of light; (LEFT) coaxial illumination (RIGHT) oblique illumination

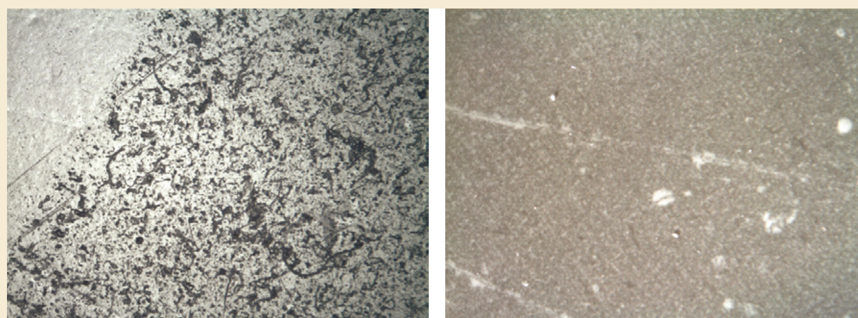
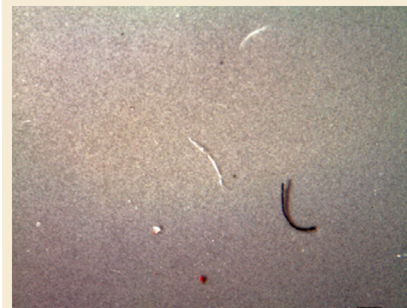


Photo 2: Filter surface with proteinaceous residue and nonprotein contaminants



FTIR and SEM/EDS Lead the Pack

In addition to light microscopy, the two most commonly used analytical techniques for analyzing biopharmaceutical contaminants are Fourier-transform infrared microspectroscopy (FTIR or micro-FTIR) and scanning electron microscopy (SEM) combined with energy-dispersive X-ray spectrometry (EDS) for elemental analysis.

FTIR can be used to identify organic materials as well as some inorganics. As substances absorb light of different frequencies they produce a unique infrared spectrum, which is a chemical "fingerprint" of each material. To prepare a sample for micro-FTIR analysis, a portion of material as small as 10 µm is isolated by hand, then pressed into a thin film and mounted on a potassium bromide crystal. A micro-FTIR system — a polarizing light microscope interfaced with an infrared spectrometer — shines a beam of infrared radiation through the sample and records the different frequencies at which it absorbs the light. By comparing the resulting spectrum with spectra of known compounds from a reference library through an automated computer search, scientists can often identify the contaminants present.

SEM/EDS provides two kinds of information: a high-quality morphological image showing the features of a contaminant and a spectrum of the elemental constituents present in a sample. EDS is commonly used to analyze inorganic materials and identify contaminants such as metals, glass fragments, and minerals. The technique also can be used to characterize certain organic materials, particularly if they contain elements other than carbon and oxygen (as do silicone rubber stopper fragments). EDS analysis may also provide a clue as to the identity of charred contaminants if a specific element is present that can be traced back to a suspected source. For example, titanium (from titanium dioxide) may suggest a white-pigmented substance.

Other Useful Microanalytical Techniques

Raman spectroscopy, a complementary technique to infrared analysis, provides "fingerprints" of many inorganic materials and other opaque samples that are unsuitable for FTIR. For instance, FTIR cannot distinguish between different crystalline forms of carbon such as graphite and amorphous char, but Raman can.

Gas or liquid chromatographic methods can be used to identify certain volatile or liquid contaminants in pharmaceutical products. In a chromatographic analysis, a sample is injected into a column, and as it travels through the column its components are separated and observed as individual peaks on a chromatogram. Mass-spectrometric detection used in combination with GC or LC analyzes the fragmentation pattern of each peak and provides a mass spectrum "fingerprint" of the unknown compound. The resultant mass spectrum is compared with reference spectra of known compounds to identify the unknown sample. The GC-MS method has been used successfully to identify plasticizers from vinyl materials and other plastic additives from packaging materials absorbed by pharmaceutical products.

Micro–X-ray diffraction (micro-XRD) is useful for identifying inorganic materials. The resulting diffraction patterns are compared with a database of known compounds. In addition to identifying contaminants, micro-XRD can also distinguish between crystalline phases (polymorphs) of active pharmaceutical ingredients.

Project Completion

Upon completing the analysis and identifying a contaminant or defect, the laboratory provides a detailed report to its customer including a

summary of the analyses performed, photographs taken, raw data (spectra), and a summary of the data evaluation and interpretation. This report provides contaminant identity and suggests possible sources based on the composition of the contaminant(s) and information gathered from discussions with the client. If a client introduces changes to its production process based on recommendations in the report, the product should be retested to ensure that the contamination problem has been eliminated.

Scientists at McCrone Associates have analyzed thousands of different products for the pharmaceutical industry. Some common contamination and defect culprits encountered include fibers from cleanroom wipes, latex glove fragments, metal wear particles from machinery, glass particles, and charred contaminants such as hair, fibers, rubber from the stopper, or latex gloves.

Continuing Education

Pharmaceutical industry quality control personnel face considerable challenges in keeping abreast not only of evolving regulatory requirements, but also the range of microanalytical capabilities available. Many educational institutions cater to this growing need. For instance, the McCrone Group's College of Microscopy teaches practica on specialized isolation and mounting techniques (COM310: Sample Preparation — Pharmaceuticals and Medical Devices) and specific analytical approaches to identifying particle contamination for regulatory compliance under FDA rules (COM410: Microscopical Identification of Pharmaceutical Materials and Contaminants).

For Further Reading

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Mary Stellmack *is a senior research chemist at McCrone Associates, Inc. (the analytical division of The McCrone Group) and an instructor at the College of Microscopy, 850 Pasquinelli Drive, Westmont, IL 60559-5539; 1-630-887-7100 ext. 2419; mstellmack@mccrone.com; www.collegeofmicroscopy.com.*