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Forensic Investigation of Biopharmaceutical Manufacturing Incidents by Light Microscopy, FTIR Microscopy, Scanning Electron Microscopy and Energy Dispersive X-ray Spectrometry

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Contaminants, such as micro-particles, found in the manufacturing stream of biopharmaceuticals must be identified and the root cause must be determined to take corrective actions. Biopharmaceuticals differ significantly from conventional pharmaceutics in manufacturing and delivery. They are manufactured by molecular cloning, cell culture fermentation, purification by multiple HPLC columns, and formulated as injectables to bypass the digestive system. Different contaminants may occur during the many steps of the manufacturing processes. For instance, the presence of contaminants in the in-coming raw materials, manufacturing equipment, drug delivery devices will result in the quarantine of materials, production slow down and even loss of product. Contaminants in cell culture media may stop cell growth and production. For final products to be delivered in liquid formulation, any visible particles are not allowed. In our lab, we employed multiple microspectroscopic techniques to investigate and identify the unknown materials that are involved in the contaminant incidents. They include but are not limited to optical microscopy, FTIR-microscopy, and scanning electron microscopy (SEM) with energy dispersive x-ray spectrometry (EDS). In this paper, we will report a few archetypical incident investigations to demonstrate the challenges and the diversity of the manufacturing incidents and the capability and the flexibility of the combination of micro-spectroscopic techniques to the biopharmaceutical manufacturing industry.

The first incident involves three syringes that had been observed to have defects and were removed from the manufacturing line (Figs. 1a, 1b and 1c). These defects, while different in appearance, all had the commonality of being between the glass barrel and the plunger stopper. Syringe 1 has a small gray and black particle that was gathered at one of the ridges of the plunger. Syringe 2 has a marbleized brown material gathered just under the edge of the ETFE coating. Syringe 3 exhibits a translucent green material on the surface of the plunger. FTIR microscopy identified the materials as nylon, protein and a polyvinyl chloride (PVC), respectively [1, 2, 3]. EDS elemental analysis confirmed these results.

The second incident resulted in cell culture fermentation where precipitates were formed during the cell culture medium holding period. The cell growth was inhibited significantly in the presence of these unknown precipitates. Each was collected and examined microscopically. Four types of crystals were observed, consisting of thin rectangles (the predominant species), rods, twisting rods, and very small amorphous particles. The thin rectangular particle was an orthorhombic ($a \neq b \neq c$) crystal that appeared slightly yellow. It exhibited parallel extinction positions in crossed polarized transmitted light (Fig. 2a). An EDS line scanning of the mixture of precipitates showed areas of pure sulfur (Fig. 2b) [4]. The combination of optical microscopy and EDS identified the predominant precipitate as orthorhombic sulfur.

The third incident occurs during the preventive maintenance of manufacturing equipment. A filter membrane was observed to contain micro-particles. Visually, there were two types of particles (Figs. 3a and 3b). The most prevalent were metallic. The third type of particle appears white to off white and opaque. Analysis of the metallic particles resulted in identification of two types of metals. One is stainless steel that contains Fe, Cr, and Ni as the major elements. The other four metal particles were identified as a nickel-chromium alloy. Analysis of the white opaque particles resulted in varying ratios of Zr, O and Al with traces of Hf and Y. The white particles are identified as a zirconium-aluminum composite ceramic [3].

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[5] We grateful acknowledge Dr. Linda O. Narhi for constructive discussion.


Fig. 1a

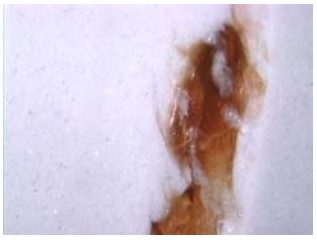
Fig. 1b


Fig. 1c

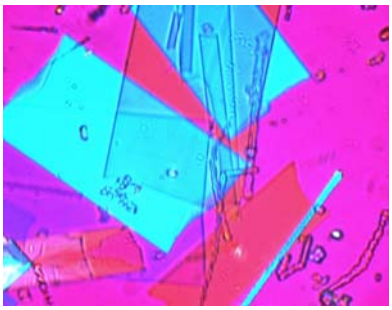
Fig. 2a

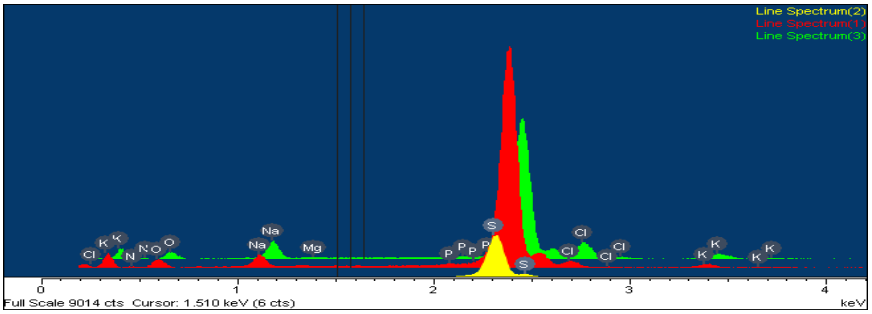

Fig. 2b

Fig. 3a

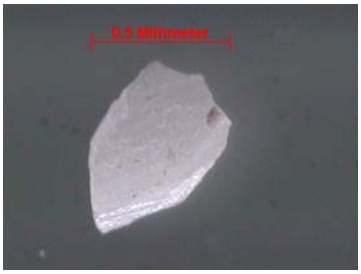

Fig. 3b

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