The Analysis of Unknown Particles Isolated from Compounded Pharmaceutical Products Using Optical Microscopy, FT-IR and Raman Micro Spectroscopy

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Abstract

There are certain instances when a medical professional may need to prescribe a patient a pharmaceutical preparation not commercially available. In this case, they may turn to a compounding pharmacy to obtain the necessary medicine. Both sterile and non-sterile products may be prepared by compounding pharmacies and in either case they require specialized equipment, facilities, training and expertise. Sterile products include ophthalmic, inhalation and injectable products while non-sterile products include solid dosage forms or topical. Although care and precautions are taken, contamination of compounded pharmaceutical products may occur. One type of contamination includes the presence of unknown particles that may come from environmental contamination, packaging components and formulation interactions and errors. The identification of these particles is important to determine the source of contamination but more importantly to protect the patient's health.

Depending on the product matrix, the selection of the proper isolation and preparation method is important to maintain the integrity of the unknown particle(s). Once they are isolated, the choice of instrumental or wet chemical analysis is important to determining their identity. Solid state analysis techniques such as optical microscopy, molecular micro spectroscopy (UV-VIS, FT-IR and Raman) and even mass spectrometry (direct analysis in real time, DART) can be used to derive useful chemical information. These techniques provide morphological and chemical information about the unknown particles which can be useful in determining their origin and allow for their retention for further analysis.

Introduction

Samples containing unknown particles submitted to the Forensic Chemistry Center (FCC) for analysis come from different sources. They may be submitted as part of criminal investigations, regulatory inspections or consumer complaints. Determining the identification of the unknown materials or particles can provide useful information to the investigator as well protect the public health from a potential hazard.

If a pharmaceutical preparation is not commercially available, a medical professional may turn to a compounding pharmacy to obtain the necessary medicine for a patient. Although care and precautions are taken when preparing compounded pharmaceutical products, unknown particle contamination may occur. Over the years, the FCC has received a variety of compounded pharmaceutical products to analyze including inhalation, ophthalmic, topical and injectable products. Many of these products are liquid-based and present a unique challenge when isolating unknown particles.

In cases of solid or semi-solid matrices, the unknown particles are fixed or localized in the product. However, a liquid matrix presents different problems when trying to isolate unknown particles for visual examination and analysis. The particles can move, float, sink, and may be difficult to see due to the product container (e.g., opaque or colored) or the refractive index of the liquid making the process difficult and time consuming.

The initial visual examination of a product is important to document the particles in the container, determine the different particle types present, their estimated size and any other characteristics which may be useful in deciding the type of sample preparation method to use. Three different sample preparation methods have been found to be the most useful: filtration, centrifugation and physical removal. Once isolated, the particles are visually examined using optical microscopy and can be analyzed using Fourier transform infrared (FT-IR) micro spectroscopy and/or Raman micro spectroscopy.

Materials and Methods

Sample Preparation Methods: Using stereo light microscopy (SLM), the sample container is visually examined using various magnifications and lighting (reflectance, transmitted or oblique). Stereoscopic photomicrographs are collected using a digital camera.

A. Filtration: The sample liquid is filtered through a PORETICS® 0.2 µm polycarbonate membrane filter. The sample container and then the filtering system is rinsed twice with reverse osmosis H2O and EtOH. The polycarbonate filter with particles is placed in a small petri dish held in place by double-sided tape. Blank samples (H_2O) are prepared before and after the sample is filtered

B. Centrifugation: A volume of sample liquid with particles is centrifuged at 10,000 RPM for 5 min. The liquid is decanted using a glass pipette. The particles are transferred to a clean vial or glass slide followed by further clean up (drying, washing etc.) if necessary.

C. Physical removal: Particles removed from liquid using tweezers, micro tools or glass pipets. Particles are transferred to clean glass vials or placed on glass slides followed by further clean up (drying, washing etc.) if necessary.

Particle Examination and Analysis Methods: The particle(s) are visually examined using optical microscopy (SLM and/or polarized light microscopy (PLM)). Photomicrographs are collected using a digital camera. Chemical analysis of particles is performed using a Fourier transform infrared (FT-IR) microscope with an attenuated total reflectance (ATR) objective and/or Raman microscope with glass objectives.

Results and Discussion

Example 1: Budesonide inhaler cartridges
The cartridges were opaque and were visually examined using SLM with transmitted light. Numerous unknown particles were observed inside the cartridges (Figs. 1A, 1B). A representative group of cartridges containing particles was individually prepared using the filtration method. Each filter was visually examined, and the different particle types noted (Figs. 1C, 1D). The white material observed on the filters was budesonide isolated during the filtering process. Selected particles were analyzed using FT-IR micro spectroscopy. The different colored fibers were determined to be semi-synthetic cellulose type fibers. Various other particles were found to contain inorganics (e.g., sulfates, carbonates, talc), polymers and cellulose materials. Of particular interest was a large particle isolated (Fig. 1E) from one cartridge. Using optical microscopy, this material exhibited the same optical and morphological properties as wood[1].

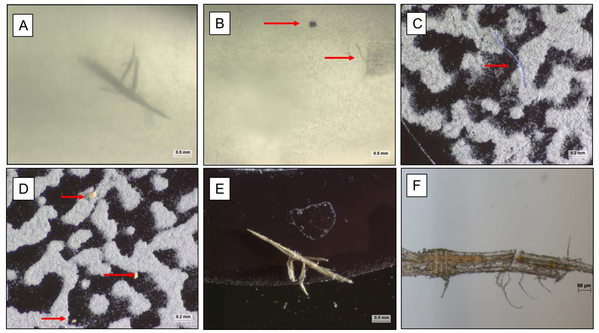

Figure 1. Stereomicroscope photomicrographs of (A) unknown material and (B) particle and fiber material observed in inhaler cartridges, (C) fiber, and (D) unknown particles isolated on filter, (E) unknown material isolated on filter, (F) a section of (E) viewed in plane polarized light.

Example 2: Testosterone topical solution
An oil-based testosterone topical solution in an amber bottle was received for analysis containing numerous unknown particles (Figs. 2A, 2B). A portion of the liquid was centrifuged to concentrate the particles, which were isolated and washed with $CHCl_3$ to remove the oil and air dried. A portion of the isolated washed and dried particles were mounted on a glass slide using Cargille oil (refractive index =1.600) and examined using optical microscopy. The unknown particles consisted of clear elongated particles and opaque irregular shaped particles (Figs. 2C, 2D). The unknown particles exhibited the same optical and morphological properties as a standard of microcrystalline cellulose[2]. Additional particles were analyzed using FT-IR micro spectroscopy and were determined to be cellulose (Figs. 2E, 2F). The arrows in the sample FT-IR spectrum indicate residual oil left on the sample after washing.

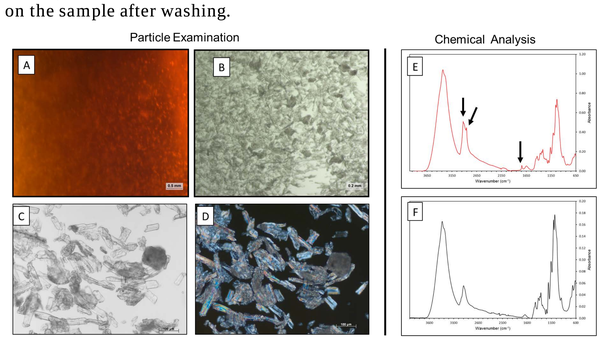

Figure 2. Stereoscopic photomicrographs of (A) unknown material observed in bottle, (B) unknown material isolated from sample using centrifuge method, (C) unknown material mounted on glass slide viewed in plane polarized light and (D) crossed polarized light, (E) FT-IR spectrum of unknown material, (F) FT-IR spectrum of a microcrystalline cellulose standard.

Example 3: Testosterone cypionate injectable liquid product
Several injectable vials were received for analysis that contained numerous white to grey amorphous unknown particles, highlighted by the black circles (Fig. 3A). The unknown particles were removed from the liquid using a glass pipet (Fig. 3B). Several particles were placed on a glass slide and compressed using a glass cover slip without mounting media. Using PLM, the unknown particles appeared to be a soft conglomerate composed of different materials (Figs. 3C, 3D) and was further analyzed using FT-IR and Raman micro spectroscopies. The FT-IR spectrum indicated the presence of poly(isobutene) (a) and talc (b) (Fig. 3E). The Raman spectrum indicated the presence of titanium dioxide (c) (Fig. 3F). Additionally, a portion of the sample stopper material from the injectable product was prepared and analyzed in the same manner as the suspect particles. The PLM, FT-IR and Raman analysis results of the unknown particles were consistent with results obtained from the injectable stopper.

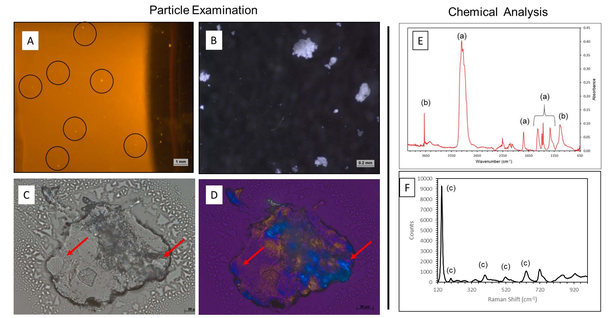

Figure 3. Stereomicroscope photomicrographs of (A) unknown particles observed in the liquid of an injectable vial, (B) particles isolated from the liquid in the vial using physical removal method, (C) unknown particle mounted on a glass slide viewed in plane polarized light and (D) crossed polarized light with a 1st order red compensator, (E) FT-IR spectrum of unknown material, (F) Raman spectrum of unknown material.

Conclusion

Compounding pharmacies take necessary precautions and care when preparing pharmaceutical products; however, contamination may occur. The examples shown demonstrate that unknown particles can come from a variety of sources including environmental, formulation errors and packaging components. Isolating and analyzing these unknown particles can be difficult, especially for liquid products. The difficulty can be minimized when using a variety of sample preparation methods. Once isolated, the unknown particles can be analyzed using techniques that require minimal sample preparation such as optical microscopy, FT-IR and Raman spectroscopy. These techniques are useful in identifying the unknown particles which can be helpful in identifying the source and potential hazard of the contamination.

References

1. R.B. Hoadley, Identifying Wood, The Taunton Press, 1990.
2. A.N. Winchell, The Optical Properties of Organic Compounds, McCrone Research Institute, 1987, 2nd ed.