Hound blasts through your problems: ID inorganic and metal particles with LIBS

Introduction

The presence of visible and subvisible particulate matter is a risk throughout the development, packaging, and delivery of biologic drugs. There are many sources of potential particulate contamination. Inherent particles, like protein aggregates, come from the formulation itself. Significant contamination risks can come from intrinsic sources such as metal fragments or filter fibers from processing equipment, or glass chips from primary packaging. Extrinsic sources like hair or clothing fibers are also contamination risks. The list of potential contaminants span protein, organic, inorganic and metal particulates.

Hound combines automated microscopy, Raman spectroscopy, and Laser Induced Breakdown Spectroscopy (LIBS) in a single instrument to identify visible and subvisible particles across a wide range of chemical compositions (Figure 1). Hound can either fully automate the identification of thousands of particles or be used manually to quickly identify a few particles. Automated microscopy can be used to rapidly count and size all particles in a sample. Raman spectroscopy is used to chemically fingerprint protein, organic, and inorganic particles. LIBS is used to perform elemental analysis to identify inorganics and metals. Particles are identified by matching the chemical and elemental fingerprints with built-in Raman and LIBS reference databases. In addition to the built-in database, custom reference spectra can be added to tailor identification to a specific process.

In this Hound application note, LIBS was used to identify the elemental composition of five inorganic contaminants: a syringe needle fragment, a bottle cap fragment, copper wire, a metal crimp cap fragment, and a glass shard. The metal cap fragment was compared to a custom reference database of items found in our laboratory to identify the exact source of the particle.

Figure 1: Hound counts and identifies the composition of visible and sub-visible particles with both automated and manual modes. Hound uses Raman (532 nm and 785 nm) and LIBS to identify the composition of particles, helping users track down the particle source.

Methods

Sample preparation

Visible particles were made by cutting or shattering materials commonly found in a laboratory. Metal particles were collected from four different sources: a common syringe needle found in the lab, a bottle cap, copper jumper wire, and a Wheaton Industries metal crimp cap. A glass particle was prepared from lead-barium glass shards. Samples were prepared by placing particles on a nitrocellulose membrane, which was then glued to an aluminum mesh backing, creating an adhesive round. The adhesive round was allowed to dry completely before particle identification.

Sample identification

The adhesive round contained five particles that were analyzed automatically with LIBS. The adhesive round was placed on Hound and the entire sample area was imaged using a 10x scanning objective.

Most particles were identified with a single LIBS measurement. Particles with an outer coating (copper wire in this case) required repeat measurements at a single spot to first burn through the outer coating, then collect a measurement for particle identification.

LIBS match criteria

Spectra from all particles were compared to the built-in LIBS reference database for identification. A match rank between the sample and the reference spectra was calculated by multiplying the Pearson correlation by 1000. A match rank >700 (out of 1000) was considered a high-quality match.

Following identification of the metal crimp cap particle, the resulting spectrum was compared to a custom reference database that contained a user-created reference spectrum for the exact Wheaton Industries crimp cap used. The spectrum from the metal crimp cap particle was re-analyzed and compared to the custom reference database in Hound Analysis.

Results

LIBS analysis

A mosaic of the filter area was imaged using the 10x objective (Figure 2), followed by identification with LIBS. Hound identifies the composition of a particle by comparing the spectrum obtained during analysis to spectra in the reference database to find a match (Table 1).

The syringe needle fragment was identified as stainless steel with a match rank of 953 when compared to the reference (Figure 3A). The bottle cap particle was identified as low alloy steel with a match rank of 860 compared to the reference (Figure 3B).

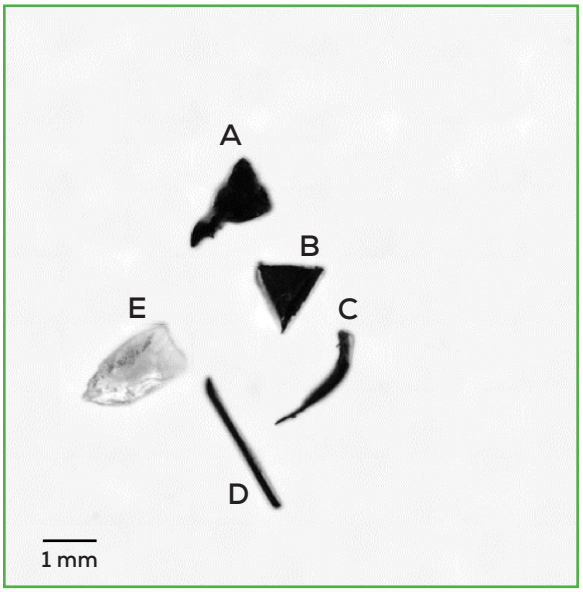

Figure 2: Mosaic image captured by automatically scanning an adhesive round at 10x. The five particles analyzed can clearly be seen. A: Syringe needle fragment. B: Crimp cap particle. C: Bottle cap fragment. D: Piece of copper wire. E: Glass shard. LIBS spectra were acquired for each particle and compared to the Hound built-in reference database.

Particle source	Match name	Match rank
Syringe needle	Stainless steel	953
Bottle cap	Low alloy steel	860
Copper jumper wire	Copper	983
Glass	Lead-barium glass	950
Crimp cap	Aluminum	993

Table 1: Each of the five particles were identified with LIBS on Hound at high match ranks. A match rank >700 (out of 1000) is considered a high-quality match.

Two repeated LIBS measurements were needed to identify the composition of the copper wire. The first measurement returned an unidentified result, but burned through the outer coating on the wire. The second measurement in the same location identified the composition of the particle as copper. The copper wire was identified as copper with a rank of 983 (Figure 3C).

The glass shard was identified as lead-barium glass with a rank of 950 (Figure 3D). The glass

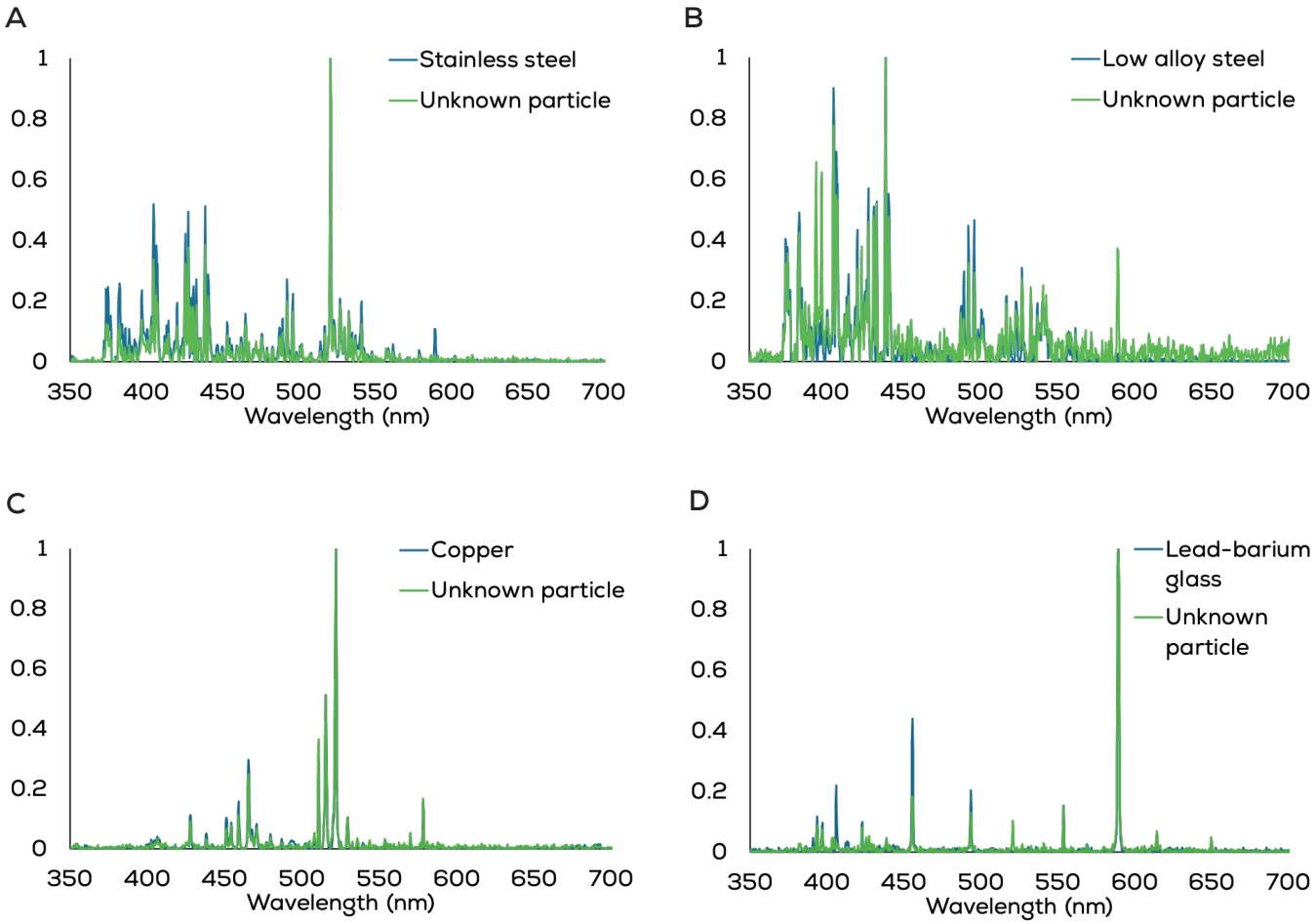

Figure 3: **A**: The LIBS spectrum (green) of a syringe needle fragment matched to a stainless-steel reference (blue) with a rank of 953. **B**: The LIBS spectrum of a bottle cap fragment (green) matched to a low alloy steel reference (blue) with a rank of 860. **C**: The LIBS spectrum of a copper jumper wire (green) matched to a copper reference (blue) with a rank of 983. **D**: The LIBS spectrum of a lead-barium glass shard (green) matched to a lead-barium glass reference (blue) with a rank of 950. All reference spectra are in the Hound built-in reference database.

shard was known to actually be lead-barium glass, confirming the accuracy of the analysis.

The metal crimp cap particle was identified as aluminum with a single measurement. The spectrum obtained from the metal crimp cap particle was a high match to the reference database spectrum for aluminum with a rank of 993 (Figure 4A).

The spectrum from the metal crimp cap was reanalyzed and compared to a custom reference database containing the LIBS spectrum for the Wheaton Industries crimp cap used to make the particle in this study. The metal crimp cap matched the Wheaton crimp cap reference spectrum with a rank of 992 out of 1000 (Figure 4B).

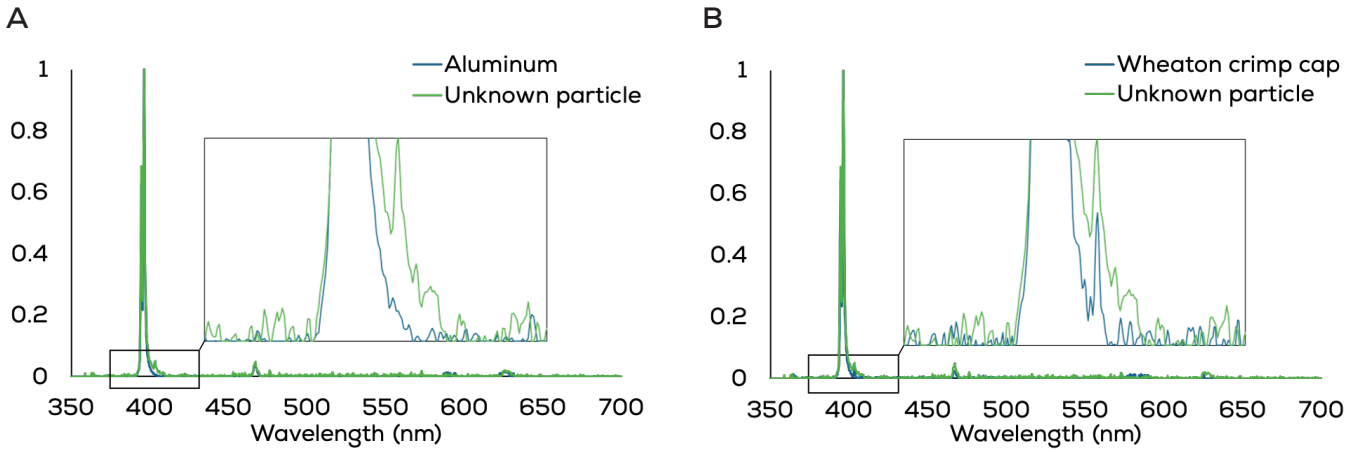

Figure 4: **A**: The LIBS spectrum of a metal crimp cap particle (green) matched to Hound's built-in reference spectrum for aluminum (blue) with a rank of 993. **B**: The spectrum of a metal crimp cap particle (green) compared to the custom mean reference spectrum for the brand of aluminum crimp cap used in this study (blue) in the custom reference database. The metal crimp cap was matched to the Wheaton crimp cap custom reference with a rank of 992. The expanded area between 375 nm and 425 nm shows a distinct peak at 403 nm for the Wheaton crimp cap.

Conclusion

Hound was able to identify the composition of all five elemental particles with LIBS. A user can obtain a particle's elemental identification to narrow down the particle source with a built-in reference database. An additional custom reference database makes it possible for the operator to create specific reference spectra for materials that are used within their laboratory or throughout their process. This enables them to identify the exact source of a particle in the event of a contamination in the future.

Unchained Labs
6870 Koll Center Parkway
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com