AAPS Interlab Study: Protocol for Visual Inspection

Introduction

Visual inspection will be used to detect and quantify the number of visible particles in vials of protein solutions, as in accordance with Pharm. Eur. Chapter 2.9.20 "Particulate Contamination: Visible Particles." The primary reported results are the visual observations of the analyst (i.e. absence/presence, number, and descriptions of particles).

Important Notes: This protocol is not a Standard Operating Protocol and assumes the required instrument is in good working order and the analysis is performed by experienced user(s). Required calibration or check standards should be run according to the manufacturer instructions. This protocol does not contain all the details of the analysis. The analyst should rely on their best judgment, routine practices, and knowledge of the technique to conduct the study; this protocol should be used as a guideline for the analysis. Samples should be analyzed immediately after thawing and preparation.

Equipment and Materials List

- Inspection station with the following features
  - A vertical matte black panel
  - A vertical non-glare white panel
  - A horizontal non-glare white panel on the bottom surface of the light box
  - Adjustable lamp holder with shaded, white light source and a diffuser, that produces illumination at the viewing point between 2,000 and 3,750 lux for clear glass ampoules.
- Calibrated light meter set to fluorescent mode
- Protein samples and formulation buffers supplied by sample originators (see Table 1)
  - Buffers, aliquots of unstressed and light stressed from Shipping #1, Tier 2 only
  - Pre-diluted stir stressed from Shipping #2
- 2 mL sterile glass vials, stoppers, and caps supplied in Shipping #2
- At least 1 inspector (more are preferred)

Reagents and Solutions

Table 1: The following samples and their buffers will be provided by sample originators. All stressed material were generated at nominally 1 mg/mL.

Proteins	Formulations	Samples
Amgen IgG2	10 mM sodium acetate, pH 5.0	Unstressed, 1 mg/mL Stir stressed, 1 mg/mL Light stressed, 1 mg/mL
NISTmAb IgG1	12.5 mM L-histidine, 12.5 mMol L-histidine HCl, pH 6.0	Unstressed, 1 mg/mL Stir stressed, 1 mg/mL Light stressed, 1 mg/mL

Procedures

Instrument Preparation

1.) Follow all instrument operating instructions to ensure appropriate use and personnel safety.
2.) Visual inspection should be performed in the inspection station using the fluorescent light setting with the diffuser.
3.) Light intensity measurements at the center of the light booth should be made using the light meter, before and after the visual inspection, with the values recorded into the accompanying Excel Sheet titled "**Visual_Inspection_Data_23082023**".
4.) Before sample preparation, inspect the empty vials for cracks, scratches, etc.

Sample Preparation

1.) Material from Shipment #1 and Shipment #2 will be used to prepare the samples for visual inspection. The 2 mL vials and pre-diluted stirred samples from Shipment #2 and the buffer, unstressed, and light stressed samples from Shipment #1 can be used to prepare the samples.
    a. The stirred samples can be prepared using the pre-diluted stirred samples sent in Shipping #2. These samples will be inspected at a 100 fold dilution from the stressed sample.
        i. For the stirred samples, 1 prediluted vial can be thawed for 30 minutes and mixed thoroughly. To mix the sample, pipet with 1 mL tip near the bottom of the sample tube 10 times in different directions but not touching the bottom or creating bubbles. Remove 1 mL of the sample from near the bottom of the vial, from about ¾ of the depth of the tube from the top of the liquid before ejecting the sample into the 2 mL glass vial provided.
    b. To prepare the buffer, unstressed, and light stressed samples for visual inspection, only use the samples allotted for **Tier 2, only if you received more than 1 mL.** . Do not dilute these samples. Since this is a non-destructive test, **you must reuse** this material for the Tier 2 methods after completing visual inspection of these samples.
    c. It is possible that not all labs will be able to inspect the unstressed and light stressed samples if they do not plan to perform the Tier 2 studies or received less than 1 mL of these samples.
2.) All vials should be visually inspected by the same analyst(s) in a single sitting.
3.) The containers should be equilibrated to room temperature for 30 minutes prior to testing.
4.) Before inspection, wipe the outside of the vial to remove moisture or marks (e.g. fingerprints). Powder free gloves are recommended when handling test articles.

Measurement

1.) If multiple analysts will perform the analysis, each analyst should report their observations in separate copies of the **Visual Inspection Data** Template. These copies can be compiled into a single document following the inspections. Review the Reporting Template for the requested information before beginning inspections.
2.) Prior to inspection, each vial is to be gently swirled and/or inverted 3-6 times to homogenize the vial content and raise any particulates that may have settled towards the bottom of the vial. Take care not to introduce air bubbles and begin visual inspection promptly.
3.) Inspect each vial individually in front of both the white and black panel (5 s each) and report all observations into the **Visual Inspection Data** Reporting Template**.**
4.) If bubbles or undissolved visible particles are observed, allow the vial to stand for an additional 5 minutes at room temperature before being inspected again. If the visible particles persist, they are to be recorded as part of the visible particulate load.
5.) If multiple particle types are observed, list the particle types observed and their features. Be sure to include notes and any other observations.

Understanding Results

Observations from visible inspection should be directly recorded in the accompanying Visual Inspection Data Template. Samples with ≤ 5 particles will be considered "essentially free from visible particles." For samples containing ≥ 5 particles, report particle number, shape, opacity, distribution, and apparent identity. If there are too many particles and the solution appears hazy or cloudy, the properties of the particles mentioned above may be difficult to see. In such a case, the analyst can mention that the solution was too "cloudy" to characterize the particles.

Troubleshooting

If sample vials appear compromised, contact sample originators.

Further Information

For any specific questions regarding this method, please contact Balakrishnan Gurusamy at Gurusamy.Balakrishnan@bms.com. Please copy aapsinterlab@nist.gov on your email.