High-throughput visible particle, turbidity and color measurement of biologics formulations

Introduction

One of the major challenges in drug development is the need for rapid formulation of biologic drug molecules. Developed in conjunction with R&D departments across the industry, Unchained Labs addresses this challenge in both vaccine and protein formulation with the freeslate system configured for biologics formulation (Figure 1).

The freeslate system provides the opportunity to expand drug developers' experimental design spaces, using less resources and material in less time than conventional methods.

Figure 1: The freeslate system configured for biologics formulation.

Streamlining visual measurement through imaging

Assessing the formation and aggregation of both sub-visible and visible particles under a variety of stress conditions is a critical step in understanding drug product stability. Sub-visible particles are assessed using analytical techniques such as micro-flow imaging, light obscuration and dynamic light scattering. Visible particles are typically assessed using a manual inspection process to determine the sample's particle count, color and turbidity. This results in data that is semi-quantitative, varies from user to user and does not provide for image capture, data retrieval or future verification, comparison and review. Not to mention the consumption of precious sample that many manual methods require.

To streamline the assessment of visual particles suspended in solutions, Unchained Labs developed a high-throughput visual inspection station (VIS) that is integrated directly into the freeslate system (Figure 2).

Through non-destructive imaging, VIS automatically:

- Counts the visible particles suspended in solution
- Assesses formulation color against known standards
- Measures the formulation turbidity using a calibrated scale
- Captures images of vial and its contents

Automation where it counts

Particle measurement involves vial handling, suspension of particles, image capture and image analysis. Using a combination of the VIS and Lab Execution and Analysis (LEA) software, a vial/plate gripper transfers a vial to the VIS. Once the vial is loaded, particles are suspended prior to image capture by spinning the vial (Figure 3). Acceleration, rotation speed, rotation time and deceleration are all set by the user and controlled by Unchained Labs' LEA software.

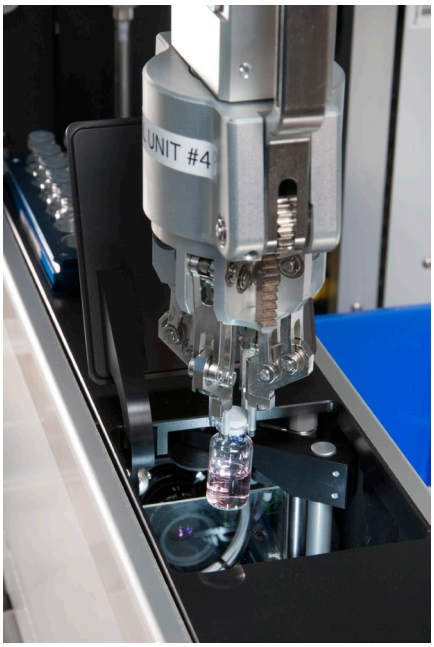

Figure 2: Unchained Labs' visual inspection station (VIS)

Spot the difference

The VIS stops the vial after spinning and captures a series of images. Successive images are subtracted to remove "static" features, including vial defects and smudges. Resultant "difference" images contain only those features (i.e., moving particles) that differ from image to image. These "difference" images contain the information about particle counts. All images are stored in the LEA database for later review and re-analysis.

The particle counting protocol

To mimic protein particles suspended in different viscosity formulations vials containing 8–9 polystyrene beads in water and approximately 55 glass beads in glycerol were analyzed. The following protocol was designed and executed to evaluate the particle counting reproducibility of the VIS.

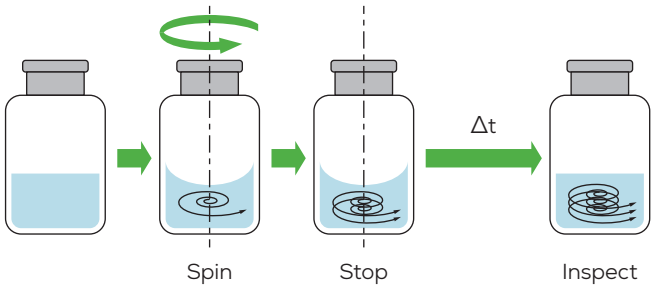

Figure 3: Mechanism of visible particle detection. Once a vial is loaded into the VIS, it spins the vial to resuspend the particles. The vial is stopped, and then the system captures a series of images.[1]

Particle counting measurement method:

1. Two 3 mL sample vials were prepared. One vial was filled with approximately 2 mL of water, the other was filled with the same volume of 65% glycerol solution. The second solution had a viscosity of approximately 15 centipoise (cP). NIST-certified standard particles were added to both vials. Approximately 55 glass beads with a diameter of 170 µm were placed in the glycerol solution and 8-9 170 µm diameter polystyrene beads were added to the vial containing water.
2. Sample vials were loaded into a plate rack, which was placed on the freeslate system.
3. The freeslate system and automatically loaded the vials into the VIS.
4. Once loaded, each vial was spun using user-specified motion settings to suspend the particles.
5. Once the spinning stopped, multiple images were acquired at a frame rate of up to 8 fps.
6. A region of interest (ROI), which is the center of the vial's image was defined.
7. Successive images were subtracted to remove static features.

8 Resultant difference images were converted to binary images.

9 Analysis was performed to identify and count particles. Both samples were measured four times. Particle counts for each measurement are presented in Table 1.

Run number	Glass beads in 65% glycerol	Polystyrene spheres in water
1	51	8
2	54	9
3	52	9
4	59	8
Manual count	50–60 particles	8–9 particles

Table 1: Particle count reproducibility results for small spherical particles in solutions.

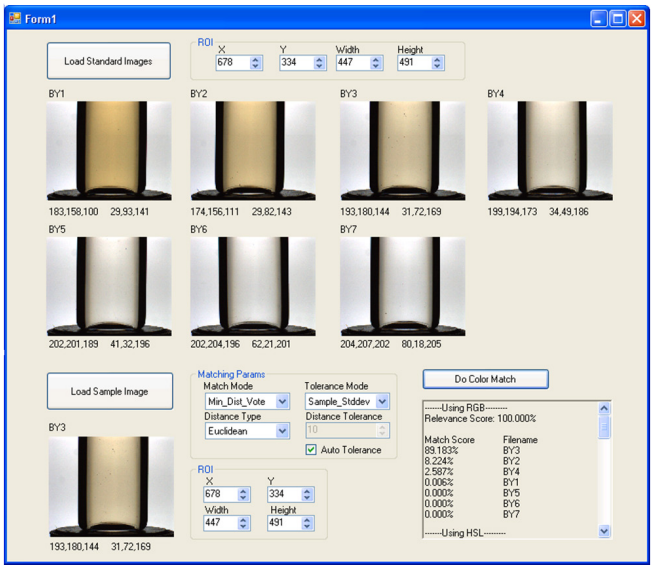

Figure 4: Color assessment of an unknown sample against a set of color standards using LEA software.

The average particle counts for repeat runs were within the expected particle count range. The run-to-run count variations are likely due to particle overlap (especially in the glycerol solution containing over 50 particles) and particles outside of the ROI during image acquisition. These results illustrate that the VIS can accurately and reproducibly count particles suspended in solutions across a range of particle sizes and a range of fluid viscosities that are relevant to drug developers.

Assessing formulation color

The VIS can also accurately assess the color of an unknown sample against a set of standards (Figure 4).

To establish reproducibility, the color of a BY4 sample in a 3 mL vial was assessed 30 times (Table 2).

Test number	Match score (%)	Match name
1	99.99	BY4
2	99.99	BY4
3	99.99	BY4
4	100.00	BY4
5	100.00	BY4
6	100.00	BY4
..	..	..
15	100.00	BY4
..	..	..
20	100.00	BY4
..	..	..
25	100.00	BY4
..	..	..
30	100.00	BY4

Table 2: Repeated color assessment of BY4 sample. All match scores between test #6 and #30 are the same.

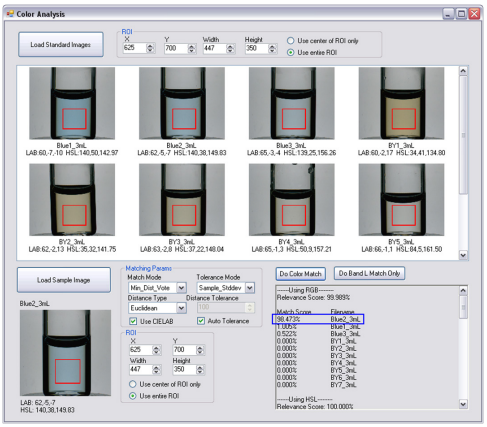

Figure 5: Blue color standards using LEA software.

The capability of the VIS to assess color across a broader array of standard colors was also evaluated. "Blue" standards were introduced among the set of color standards and then assessed using the VIS, which easily achieved a color match (Figure 5).

Non-destructive turbidity measurement

Turbidity is often a challenging measurement as it typically requires significant manual involvement and requires the use of optical cuvettes, which consume samples. To evaluate the accuracy and reproducibility of VIS for turbidity, the system measured known turbidity standards (from GFS Chemicals).

Turbid samples scatter light, which causes the average luminance of a sample to decrease compared to a low turbidity sample. The VIS utilizes a calibration curve obtained from turbidity standards to calculate the turbidity of a sample from the average measured luminance value. Each calibration curve must be generated using the same type of vial that will be used in a particular experiment.

The VIS employs a normalization method to ensure the reproducibility of turbidity measurements. The VIS normalizes luminance by acquiring two images: a "blank" image immediately prior to loading a sample and a "sample" image once the vial is in place. The luminance of this "blank" image is used to normalize the turbidity measurement using the following formula:

$$\text{Normalized luminance} = \Delta L / L = (L_{blank} - L_{sample}) / L_{blank}$$

Turbidity measurement method

1. Turbidity standards were placed in 3 mL and 20 mL vials and loaded into the VIS automatically.
2. A blank image (no vial in place) was acquired.
3. The sample vial was loaded into the VIS system.
4. A sample image was acquired.
5. The normalized luminance was calculated.
6. Steps 2–5 were repeated five times with each standard.
7. The average normalized luminance values were determined for each standard.
8. A calibration curve was generated for each vial size.

The standards used were 1, 2, 5, 10, 20, 40 and 100 Nephelometric Turbidity Units (NTU). The calibration curves are shown in Figure 6.

Reproducibility of turbidity measurements was evaulated by testing a series of turbidity standards in 3 mL vials (i.e., from 1-100 NTU) seven times. Measurements were then repeated the next day (Figure 7). Results show that all measurements were within 5% of the expected values.

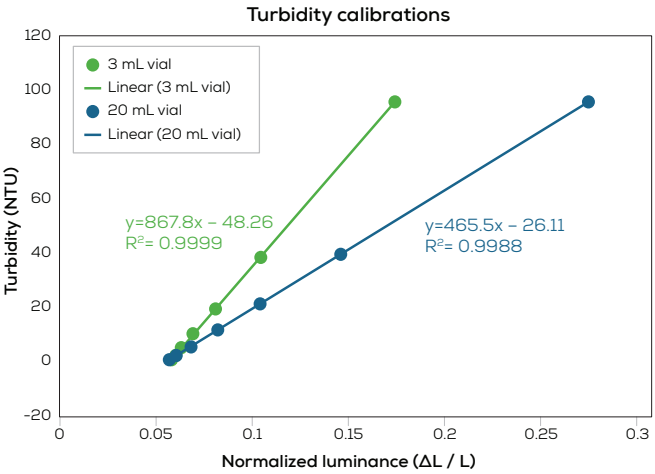

Figure 6: Turbidity calibrations for 3 mL and 20 mL vials.

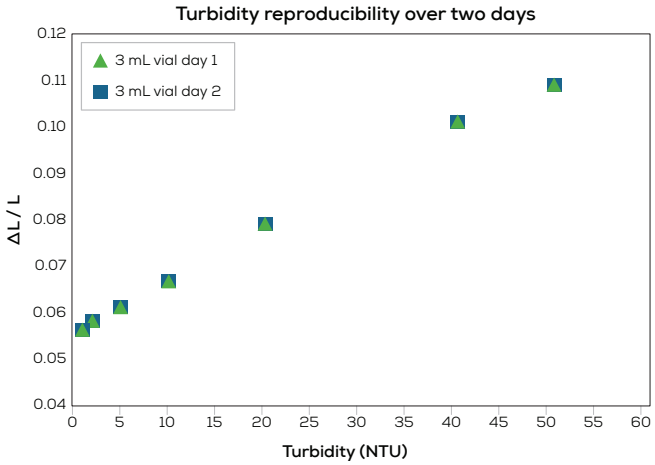

Figure 7: Turbidity measurement reproducibility data.

Conclusion

Unchained Labs' VIS was evaluated as a high-throughput device for three visual inspection applications—particle counting, color assessment and turbidity. All measurements were automated, quantitative and non-destructive.

- For particle counting, the VIS accurately and reproducibly counted particles suspended in each solution across a range of particles and solution viscosities.
- For color assessment, the VIS reliably matched colors of samples across a broad range of standards.
- For turbidity measurement, the VIS accurately and reproducibly measured samples from 5-100 NTU.

Overall, the VIS significantly increased throughput compared to labor intensive, manual visual inspection methods while maintaining high accuracy and reproducibility. The VIS further enhances the speed of measuring color, turbidity and visible particles present in liquid protein formulations leading to significant gains in productivity in formulation development.

References

1 Challenges and Strategies for Implementing Automated Visual Inspection for Biopharmaceuticals, Rathore et al., *Pharmaceutical Technology*, Nov 1, 2009

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