Fast and automated turbidity measurement of protein formulations

Introduction

Protein formulation is no easy task. Developing a safe, effective and stable formulation can require screening a wide array of formulations with a variety of analytical techniques, requiring significant hands-on time. Turbidity measurement is a simple, method routinely used to evaluate protein stability throughout formulation development. While turbidimeters are easy and accurate, they require significant hands-on time. Each sample must be manually transferred to a sample cell that must be cleaned and polished prior to analysis. This manual method is not ideal for efficiently screening large numbers of protein formulations. A better option is the visual inspection station (VIS) from Unchained Labs.

The VIS automatically measures turbidity, counts visible particles and assesses color of formulation samples. With this system, hundreds of turbidity measurements can be performed per day with minimal hands-on time. The VIS can be integrated with other analytics such as pH and viscosity on the freeslate jr. or into a customized larger workflow on a freeslate system configured for biologics formulation. In this application note, we compare the automated VIS to a standard turbidimeter, the Hach Lange 2100 AN, using five mAb drug formulations currently in development.

Materials and methods

Manual turbidity measurements were performed on the Hach Lange 2100 AN turbidimeter per user guide instructions. Each sample was analyzed at three different vial positions to minimize the interference of scratches and imperfections on the sample cell. The lowest turbidity measurement of the three positions was recorded. A freeslate jr. with the VIS was used for automated turbidity measurements. The samples and standards were loaded onto the freeslate jr. The system was programmed to perform calibration and measure the samples. The freeslate jr. then transferred each standard and sample vial into the VIS where the turbidity was measured in a specified region of the vial. The freeslate jr. then rotated the vial 90° and a second turbidity measurement performed and the readings averaged. Five replicates were measured for each sample. For direct comparison with the Hach procedure, the lowest turbidity value from the five replicate measurement was reported as the final result.

The visual inspection station can provide up to 45-fold savings of operator time for turbidity measurements of drug products.

Results

Turbidity results for the five mAb formulations are summarized in Figure 1. Turbidity results from the VIS were slightly higher but still comparable to the Hach Lange 2100 AN turbidimeter. The replicate measurements for the VIS demonstrate that data generated by the instrument are precise (Table 1).

While the two systems provide comparable results, the required hands-on time to run the systems are very different. After a short manual setup, all measurements with the VIS were unattended. The VIS requires a short system setup, about 15-20 minutes, that was performed only once for each

sequence. Typical sample sequences can range from 1–260 vials. Turbidity analysis with the VIS requires approximately two minutes per sample which includes both vial transfer and measurement. The Hach Lange 2100 AN turbidimeter measurements require about 3.5 minutes per sample. Table 2 compares the hands-on time per sample for both methods. Table 3 compares total analysis time of both methods. Using the Hach Lange 2100 AN turbidimeter to measure the turbidities of 260 samples would require approximately 15 hours of manual operation. In contrast, one scientist using Unchained Labs' instrument can measure turbidities of up to 260 samples with only a 20 minute setup providing a 45-fold savings of operator time for turbidity measurements of drug products.

mAb formulation	Turbidity (NTU)	Average turbidity (NTU)	RSD turbidity (%)	Minimum turbidity result (NTU)
A	9.45	10.0	5	9.4
	9.71			
	9.99			
	10.64			
	10.12			
B	15.71	16.6	4	15.7
	16.21			
	17.59			
	16.79			
	16.78			
C	17.48	17.9	3	17.4
	18.15			
	18.15			
	17.35			
	18.50			
D	26.79	26.7	2	26.1
	26.47			
	27.44			
	26.76			
	26.08			
E	40.02	40.9	2	40.0
	40.52			
	40.39			
	40.97			
	42.53			

Table 1: Turbidity results of five mAb formulations measured using the VIS.

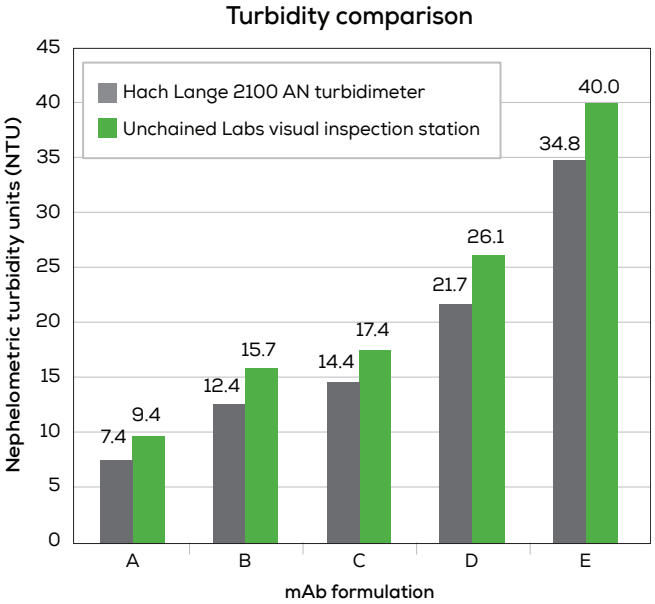

Figure 1: A comparison of the turbidity results of five mAb formulations using the Hach Lange 2100 AN turbidimeter and Unchained Labs' VIS.

Hands-on time per sample measurement	Hach	Unchained Labs
Transfer sample to cuvette	0.5 min	N/A
Polish cuvette	1 min	N/A
Read cuvette	2 min	N/A
Total time	3.5 min	0 min

Table 2: Comparison of hands-on time for Hach and Unchained Labs' turbidity instruments. After a brief setup time, turbidity analysis on the VIS is completely automated.

Hands-on time per sample measurement	Hach Required hands-on time	Unchained Labs Required hands-on time	Required Automated analysis time
Setup time	20 min	20 min	N/A
Time per sample	3.5 min	0 min	2 min
Number of samples	260	260	260
Total time	15 hrs	0.3 hrs	8.7 hrs

Table 3: Comparison of total analysis time for Hach and Unchained Labs' turbidity instruments. To automatically analyze 260 formulations with the VIS requires a 20 minute setup, and the entire analysis is complete in approximately nine hours.

Conclusion

This turbidity study demonstrates that the VIS from Unchained Labs generates comparable turbidity results faster and more easily than traditional, manual methods. When combined with the ability to automatically count visible particles and assess color changes in a formulation, the VIS allows a single scientist to quickly screen a large number of formulations with minimal manual intervention. Automating manual assays such as turbidity measurement, visible particle counting, pH measurement and viscosity can have a significant impact on lab productivity and accelerate formulation development.

Unchained Labs
6940 Koll Center Pkwy, Suite 200
Pleasanton, CA 94566
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

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