Automated Particle Analysis of Viscous Samples with MFI and the Bot1 Autosampler

Introduction

Micro-Flow Imaging™ (MFI) easily detects particle size and morphology on a wide range of particle contaminants. And when you add the Bot 1 Autosampler to your MFI 5000 Series system, you've got the automated go-to method of choice for particle analysis today. Together, this combination lets you quickly screen for any changes in levels of particle contaminants like protein aggregates and silicone oil in your biopharmaceutical formulation.

Highly concentrated protein solutions can be a little trickier to analyze with other technologies since they often need dilution due to high viscosity and/or high particulate levels.[1,2] MFI™ often lets you skip the dilution thanks to its direct image-based detection — so you can get accurate and sensitive measurement of particle size, count, and morphology in your viscous samples straight up. In this application note, we studied how the Bot1 handled viscous samples, and found that with just a few simple optimization steps, you can be running viscous samples in no time at all.

Some Great Tips for Handling Viscous Samples

We recently ran a study for viscous samples in manual mode with the MFI 5200 system[3] and found these are the hands-down best practices for handling viscous samples that apply to both the manual and automated mode with the Bot1:

Calibrate your pump — Make sure your pump is properly calibrated with water per the MVSS calibration wizard.

Calibrate your inlet port — Check the flow rates of your inlet port and pump and make sure they're synced and can transfer water without running dry or overflowing.

Flush slowly — If you're going to flush with viscous solutions, make sure to use a slow flush rate to move the matrix through the system. You'll get way more accurate results.

Mix gently — You want to mix samples well but not too aggressively, as viscous samples have a tendency to retain air bubbles. You'll also want to create a filter for air bubbles in MVAS to make sure they don't distort your particle counts.

Setting Up your MFI + Bot1 System for Viscous Samples

To get a handle on how viscous solutions affect the performance of the Bot1 Autosampler, we prepared solutions containing Polyethylene glycol (PEG). By titrating the PEG, we were able to control the viscosity of the solution and create a perfect cohort of samples ranging from 1-20 cP.

Before starting analysis, we first made sure our instrument was functioning properly by confirming the pump calibration on our MFI 5200 system was correct. We were pleased to find that the standard water calibration you'd normally use works with viscosities up to 20 cP too. So if

you've already calibrated your pump, there's no need to do it again.

Next, we made sure the dispense rate of liquid from the Bot1 pipettor was matched with the entry rate of liquid into the inlet port. This avoids any overflow of liquid out of the inlet port (inlet overflow) or too little liquid in the fluid path, as both can cause the problems with analysis during a run.

VISCOUS SOLUTION FLUIDICS

Next we looked at how the slower movement of viscous samples through the fluid path of the instrument compared to aqueous samples, and how it might impact our results. In the first set of tests, the flush volume recovered from the instrument under a slow analysis speed of 0.2 mL/min wasn't affected by viscosity (**Figure 1**, left), and gave consistent sample recovery. Under a higher flush speed of 6 mL/min, the recovery volume decreased as the sample viscosity increased (**Figure 1**, right).

To get around this, your best bet with a viscous solution is to separate the cleaning and equilibration functions of the flush process by using different flush speeds for each. So what's the best way to properly flush the flow cell before analysis? To dig into this question, we compared the impact of different flush rates on the volume recovered from the inlet port **(Figure 2)**, and found that 0.2 mL/min was optimal because it gave equivalent results to water.

So if you're planning on using a viscous solution for a flush step or to equilibrate the optical properties of the flow cell, just use a slower flow rate for the flush. If you try to flush extremely viscous solutions at high speeds, the inlet port will overflow, which you want to avoid.

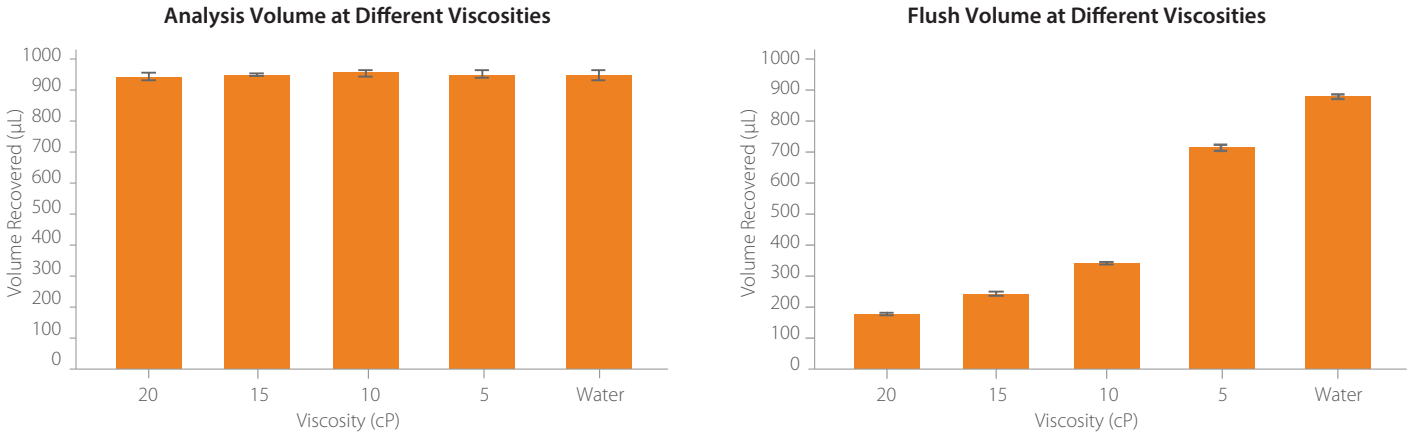

FIGURE 1. Effect of flush speed on the amount of volume recovered from viscous samples.

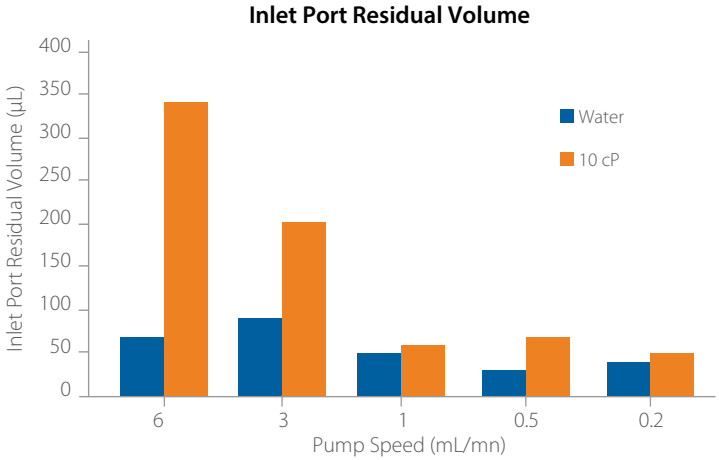

FIGURE 2. Slower flush rates are better for viscous solutions.

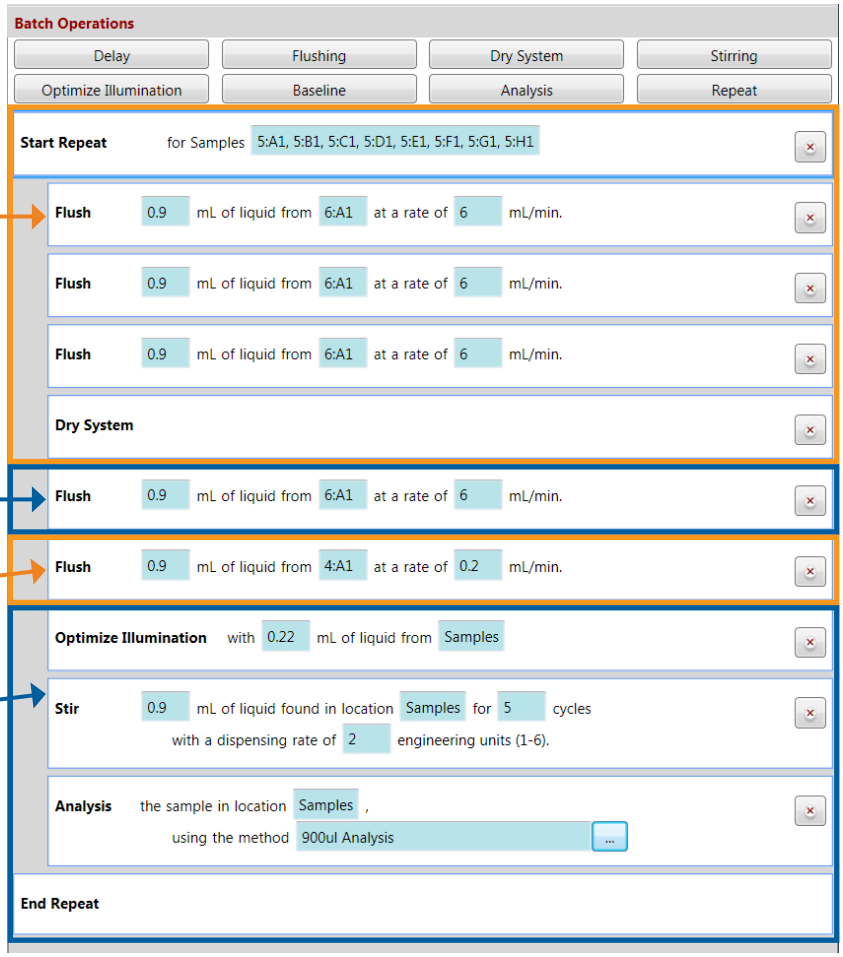

FIGURE 3. An example batch protocol for running viscous samples.

Next we applied these same concepts to the automated batch protocol. We used a faster flush step initially with aqueous buffer to really clean the flow cell, followed by a much slower flush step to equilibrate the fluid path with the viscous sample and avoid introducing Schlieren lines **(Figure 3)**. Then we added an optimize illumination step, which handles the sample at the same slow flush rate. Finally, we ran the analysis method, which by default operates at the same slow pump speed. Using this batch protocol makes sure the pump will operate at a slow speed whenever viscous solution moves through the fluid path.

The batch protocol for our viscous solutions used one flush at the max speed (6 mL/min) after the dry step to make sure any bubbles were removed from the flow cell (Removing Bubbles, **Figure 3)**. When that was done, we had free reign to change the flush speed to a lower speed that would suit the viscosity (Equilibration Step, **Figure 3**).

Mixing Conditions for Viscous Samples

Next we took a look at how mixing speed and the number of mixes impacted data quality so we could better optimize our protocol for viscosity. We found that using a slow mixing speed below 3 mL/min was essential to avoid the introduction of air bubbles when mixing a 20 cP solution. At this slow speed, any number of mixes could be used without causing bubbles to form. But, keep in mind when you're mixing a protein solution that the mixing doesn't lead to more protein aggregation.

We also wanted to find out if automated pipetting with the Bot1 increased the risk for introducing bubbles into viscous samples, so we created a particle characterization filter in MVAS to isolate them. Choosing particles that had high values for circularity and aspect ratio as well as low intensity standard deviation helped differentiate air bubbles from other particle contaminants in our labware.

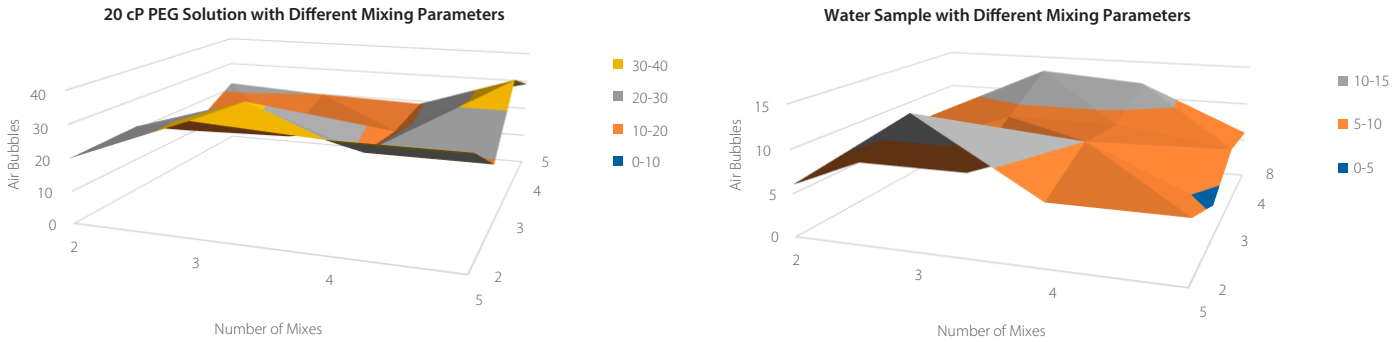

FIGURE 4. A highly viscous PEG (20 cP) solution (left) has a greater tendency to retain air bubbles than water (right). For all mixing conditions, the 20 cP solution showed higher levels of air bubbles than the water sample.

The data in **Figure 4** shows that a highly viscous (20 cP) solution is more likely to retain air bubbles than water. There was also little correlation between the number of mixes and the intensity of the mixes, so this suggests bubbles were introduced during sample preparation and not during Bot1 mixing. Literature references also suggest degassing of viscous solutions before sampling to limit the introduction of air bubbles.[2]

Comparing these different mixing conditions to our standard mixing condition (five mixes at a speed of 3 mL/min), we saw a high level of consistency in both the water and the 20 cP PEG solution **(Figure 5)**. In fact, the viscous sample results clustered around the 100% recovery level better than the water sample, possibly because the viscous matrix helped the particles to stay in suspension.

MFI Performance with Viscous Samples

To really learn how MFI system performance was affected by samples of different viscosities, we prepared NIST sizing standards in PEG solutions ranging from 1–20 cP. The final concentration of the standards was 10,000 beads/mL. The samples were plated in quadruplicate and the analysis volume for each well was set for 900 mL in the method. If you want to learn more about method parameters for viscous samples, check out the experiments we ran using the MFI 5200 system with manual injections.[3]

If you're using an MFI 5100 system with the Bot1, note that the slower flush rate is also needed for viscous solutions, although results for inlet port residual volume versus pump speed will be slightly different compared to an MFI

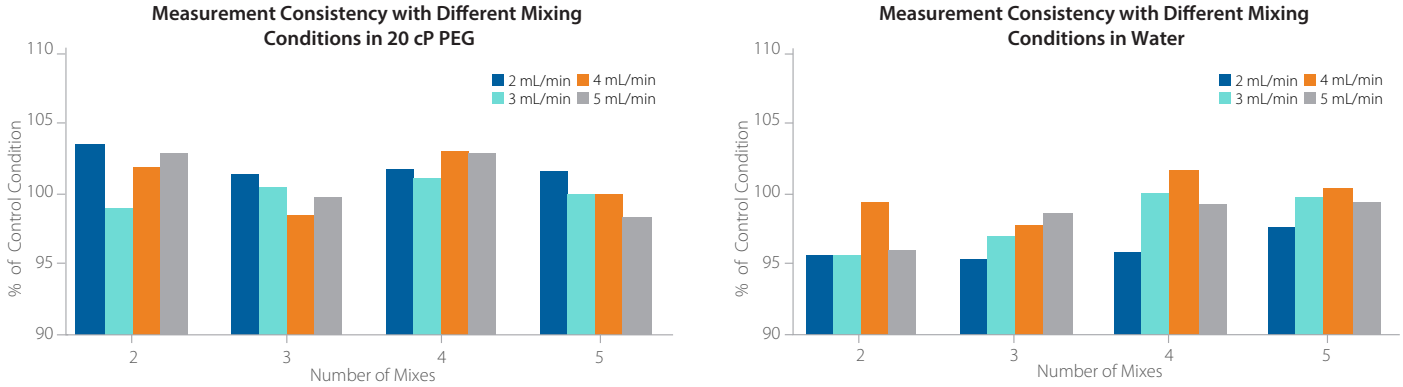

FIGURE 5. A 20 cP PEG solution (left) displays more consistent results compared to water (right) across different mixing speeds and number of mixes.

5200 with Bot1. Also, the size and morphology results for calibration beads at 2–5 microns will be slightly less accurate on an MFI 5100 compared to the MFI 5200, with or without a viscous solution.[4]

The results with the automated method demonstrated that intra-assay precision, accuracy, and particle concentration were very consistent for a broad range of viscosities. And we also confirmed that the flush speed chosen for analysis was optimized for the volume **(Figure 8)**. So what's the final word? Our results showed that the MFI 5200 system with the Bot1 operates just as well with highly viscous samples as it does with aqueous samples. For intra-assay precision, measurements from the averaged replicates gave us great %CVs at below 10% for all conditions **(Figure 6)**. And on top of that, inter-assay precision %CVs over three separate days were only 7–10.65% **(Figure 6)**. So that pretty much confirms you'll be able to get very precise data no matter how viscous your samples are.

When we compared the expected bead concentration to the actual measurements, our results correlated really well for all conditions — between 100–104% **(Figure 7)**. The nominal value for the concentration was 10,000 beads/mL.

Bead counts from the viscous samples were also very consistent, and compared well with the water control **(Figure 8)**. The water control counted 8000 beads present in the sample volume, and the viscous sample reported bead counts within 5% of that value. Since the volume recovered for viscous and water samples was equivalent, these results confirm that the MFI 5200 system with the Bot 1 measures the right volume of sample across the different viscosities.

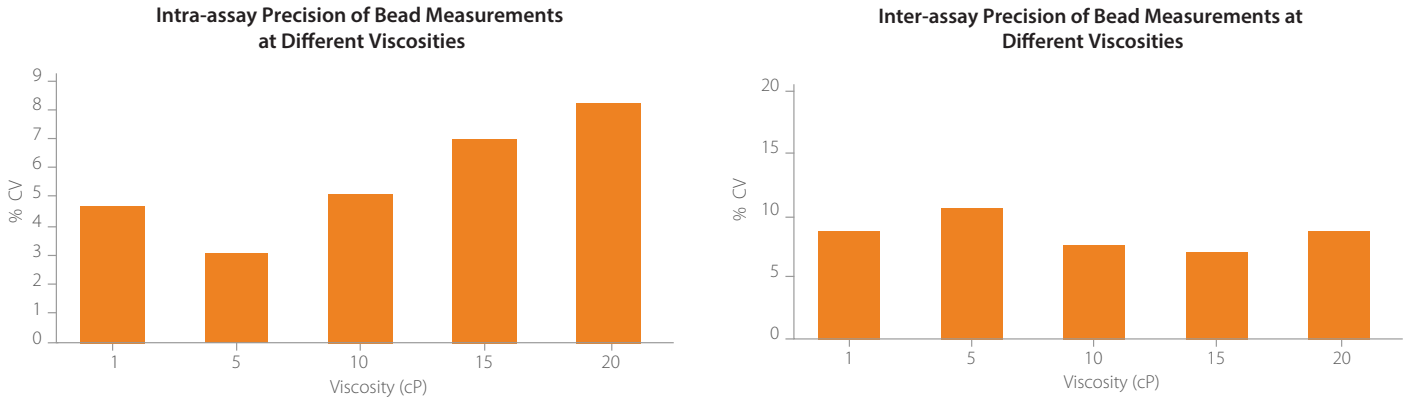

FIGURE 7. Precise bead concentration measurements even for a 20 cP PEG solution. Intra-assay %CVs were all less than 9% (left) and inter-assay %CVs were all less than 11% (right).

FIGURE 8. Data accuracy correlation between expected bead concentrations. Actual measurements were between 100–104%.

FIGURE 6. Data correlation between expected bead counts. Counts were within 5% of the control measurement.

Conclusion

Well you guessed it — MFI 5000 Series systems with the Bot1 Autosampler will give you the same fantastic data with viscous samples as it does with aqueous ones! Our studies showed really robust, reproducible performance for viscous matrices up to 20 cP. The intra-assay precision was great with replicate %CVs under 10%. And when we looked at the recovered concentration of beads in samples, there was less than a 5% difference between an aqueous sample and a 20 cP PEG solution, which confirms your measurement accuracy won't be impacted by viscosity. When we looked at the particle counts in our samples, the difference between the viscous samples and the aqueous control was well within 5%. That means when you analyze viscous samples you'll get the same great sampling efficiency up to 20 cP. Not to mention you'll get the same, consistent results day to day to boot. When we ran our cohort of viscous samples across multiple days, we got %CVs of 11% or better regardless of the viscosity.

Setting the system up is really simple too — you just need to make a few tweaks to your typical method. And if your pump is already calibrated with water, you won't have to do it again — we found that the water-based calibration works just as well for viscous samples too. So, just like you can with manual mode on an MFI system, you can also run viscous samples straight up with the Bot1 and keep the same precise and accurate particle characterization you're used to.

References

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