

Application Note: Small sample viscosity measurements using 20 microliter sample volumes with the RheoSense *m*-VROC viscometer

RheoSense Inc.’s *m*-VROC viscometer reliably and accurately measures viscosity with only 50 microliters of sample during every day lab use. This capability significantly helps reduce the material costs incurred when testing expensive samples or preserves very limited material. This is a capability unmatched in the industry.

However, in those cases where extremely small sample volumes are needed, the *m*-VROC viscometer can be used to make reliable and accurate viscosity measurements with sample volumes as low as 20 microliters. This application note describes a test method which enables viscosity measurement for these sample volumes. Such a small sample volume test affords viscosity measurement for expensive and limited early stage drugs. Other products on the market today require nearly four times as much sample to perform a viscosity measurement and most need in excess of 1 milliliter.

Constraints of micro-volume measurements

When making viscosity measurements with micro-volume sample levels, high shear rate measurements cannot be made as the equipment cannot ramp up the shear rate in that small a volume range. Therefore, micro-volume measurements are limited to shear rates up to 5,000 s⁻¹.

Recommended *m*-VROC accessories

The following accessories are recommended by RheoSense to properly execute the micro-volume measurements.

Pipette (PN: H-100-2)

100ul Zero Dead Volume Syringe (PN: VROC-300-100-0)

Compressed Gas Coupler (PN: VROC-800-4)

How to minimize sample volume with the *m*-VROC

The key to very small sample volume measurements with the *m*-VROC are proper and thorough cleaning and preparation of the VROC cell along with careful loading of the sample liquid. The test procedure below explains how to prepare the VROC Cell and the sample material for small sample volume viscosity measurement. The standard *m*-VROC procedure is used with several additional steps.

VROC Cell cleaning and preparation

Perform the following steps to properly prepare the VROC cell:

1. Flush the VROC cell with the appropriate cleaning agent.
 - a. Select the appropriate cleaning agent based upon the material previously measured. (1% Aqueet solution works well to clean the VROC cell for many protein solutions. However, you should use the solution best suited to your actual protein
 - b. Measure the viscosity of the fluid during the flushing process.
 - c. Continue flushing the VROC cell until the measured viscosity matches the standard value expected for the cleaning fluid. This indicates that all previous materials have been removed.

Example:

For BSA solutions, following cleaning protocol works well. With 500 ul syringe, run twice the cleaning protocol below.

Flow rate (µl/min)	Meas. Time (s)	Pause time (s)	Volume (µl)
200	140	1	500

2. Thoroughly dry the VROC cell
 - a. Connect the Compressed Gas Coupler, to any pressurized air or inert gas line to the inflow port of the coupler (Figure 1). Make sure to use a very clean dry air or N₂ gas and set the output pressure at 1 psi.

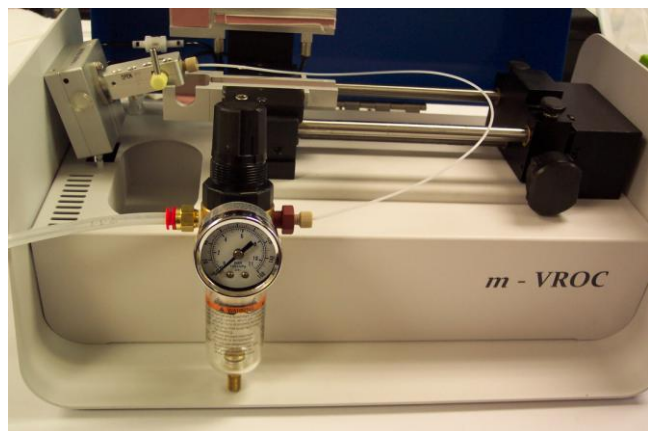


Figure 1: Compressed Gas Coupler Connection

- b. Connect the outflow port of the Compressed Gas Coupler into the VROC cell valve inlet. Start the N₂ flow and dry the chip for five minutes.
 - c. After five minutes, turn off the N₂ flow and remove the Compressed Gas Coupler from the VROC cell.

The VROC cell is now dry and ready.

Sample preparation

After the chip is thoroughly cleaned and dried, the following steps are recommended for sample preparation and measurement. RheoSense recommends use of the zero dead volume syringe to achieve the 20ul volume level but a standard 100ul syringe can be used with the sacrifice of the sample left in the syringe at full compression.

Specific methods for micro sample loading of a syringe are provided in RheoSense applications note 15; "[Avoiding Air Bubble Entrapment](#)".

1. Load the sample material into the syringe
 - a. Select the 100ul syringe
 - b. Carefully load 20ul of sample into the syringe paying special attention to prevent any air bubbles from entering the syringe.
 - c. Visually inspect the syringe and sample to ensure no air bubbles are trapped in the syringe body or tip.

Perform the measurement

1. Connect the 100ul syringe to the VROC cell using the normal procedure. Be careful not to allow any air bubbles to enter the syringe during this step.
2. Position the *m*-VROC pusher block using the normal procedure. Please make sure that the pusher block of the pump is in contact with the plunger of the syringe. Otherwise, the actual dispensed volume will be less than the reported dispensed volume.
3. Wait for 3 minutes for the sample temperature to equal the syringe jacket temperature.
4. Prime the VROC cell channel by injecting 12ul of sample into the VROC cell. This is the volume of the tube/valve fittings and VROC channel. Do this using the *m*-VROC Control software:
 - a. Select the 100ul syringe option
 - b. Select 'MANUAL' for both Meas. Time and Pause time. If the estimated viscosity is higher than 20 cp, choose a flow rate lower than 100 ul/min. For example, if the viscosity is 40cp, then the corresponding flow rate should be set to 50ul/min.
 - c. Enter the following settings into the *m*-VROC Control software to dispense 12ul into the VROC channel:

Flow rate (µl/min)	Meas. Time (s)	Pause time (s)	Volume (µl)
100	6	1	12

5. Make the viscosity measurement.
- Enter the following settings into the *m-VROC* software:

Flow rate ($\mu\text{l}/\text{min}$)	Meas. Time (s)	Pause time (s)	Volume (μl)
100	1.1	1	3.5

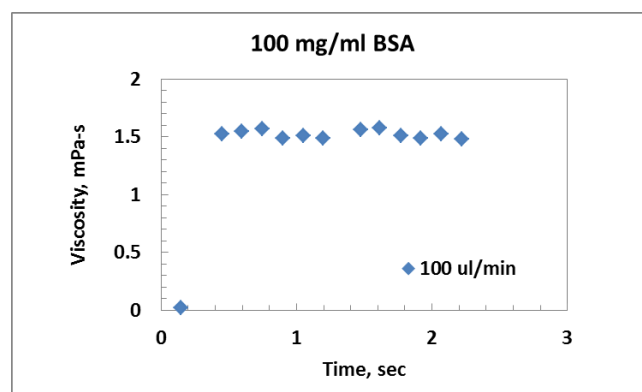
- Confirm that the viscosity measurement is valid by checking that the R^2 number displayed in the *m-VROC* software is 0.99 or greater.
- If the R^2 number displayed in the *m-VROC* software is less than 0.99, then it may be that the VROC cell channel was not fully primed during Step 4. Repeat steps 5.a and 5.b to get a good measurement.
- Alternatively, if the R^2 number is 0.99 or greater on the first run, then a second viscosity measurement can be made, repeating steps 5.a and 5.b with the remaining sample volume.

Example Measurement Results

For demonstration purposes, a 100 mg/ml BSA solution was tested. The first step is to fill the flow channel of the sensor with BSA solution. Since the swept volume of tube/valve and fittings is 7.5 μl , 12 μl dispense is enough to fill the flow channel. Please make sure that the pusher block of the pump is in contact with the plunger of the syringe. Otherwise, the actual dispensed volume will be less than the reported dispensed volume.

Flow rate, $\mu\text{l}/\text{min}$	Meas. Time, s	Pause time, s	Volume, μl	Viscosity, mPa-s
100	6	1	12	-
100	1.1	1	3.5	~1.49
100	1.1	1	3.5	1.50
100	1.1	1	3.5	1.50
200	1.1	1	3.5	1.50

The graph represents the data of the third row in the list. The graph below shows expected viscosity values measured with time once the flow channel is filled prior to viscosity measurement.



The test protocol above suggests that viscosity can be measured for sample volume as small as 17 μl . However, 20 μl of sample is recommended due to variations that occur between individual measurements.

Practice and familiarity with the instrument is also essential for successful and error free measurements.

If you have questions or need more information about this product or other applications, please contact us:

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