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TITLE:

Calibration Procedures for Orthogonal Superposition Rheology

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rheology, viscosity, calibration, Newtonian fluids, orthogonal superposition

SUMMARY:

We present a detailed calibration protocol for a commercial orthogonal superposition rheology technique using Newtonian fluids including end-effect correction factor determination methods and recommendations for best practices to reduce experimental error.

ABSTRACT:

Orthogonal superposition (OSP) rheology is an advanced rheological technique that involves superimposing a small-amplitude oscillatory shear deformation orthogonal to a primary shear flow. This technique allows the measurement of structural dynamics of complex fluids under non-linear flow conditions, which is important for the understanding and prediction of the performance of a wide range of complex fluids. The OSP rheological technique has a long history of development since the 1960s, mainly through the custom-built devices that highlighted the power of this technique. The OSP technique is now commercially available to the rheology community. Given the complicated design of the OSP geometry and the non-ideal flow field, users should understand the magnitude and sources of measurement error. This study presents calibration procedures using Newtonian fluids that includes recommendations for best practices to reduce measurement errors. Specifically, detailed information on the end-effect factor determination method, sample filling procedure, and identification of the appropriate measurement range (e.g., shear rate, frequency, etc.) are provided.

INTRODUCTION:

Understanding the rheological properties of complex fluids is essential to many industries for the development and manufacture of reliable and reproducible products[1]. These “complex fluids” include suspensions, polymeric liquids, and foams that widely exist in our everyday life, for example, in personal care products, foods, cosmetics, and household products. The rheological or flow properties (e.g., viscosity) are a key quantity of interest in establishing performance metrics for end use and processability, but flow properties are interconnected with the microstructures that exist within complex fluids. One prominent characteristic of complex fluids that distinguishes them from simple liquids is that they possess diverse microstructures spanning multiple length scales[2]. Those microstructures can be easily affected by different flow conditions, which, in turn, result in changes in their macroscopic properties. Unlocking this structure-property loop via non-linear viscoelastic behavior of complex fluids in response to flow and deformation remains a challenging task for experimental rheologists.

Orthogonal superposition (OSP) rheology[3] is a robust technique to address this measurement challenge. In this technique, a small amplitude oscillatory shear flow is superimposed orthogonally to a unidirectional primary steady-shear flow, which enables the simultaneous measurement of a viscoelastic relaxation spectrum under the imposed primary shear flow. To be more specific, the small oscillatory shear perturbation can be analyzed using theories in linear viscoelasticity[4], while the non-linear flow condition is achieved by the primary steady-shear flow. As the two flow fields are orthogonal and thus not coupled, the perturbation spectra can be directly related to the variation of the microstructure under the primary non-linear flow[5]. This advanced measurement technique offers an opportunity to elucidate structure-property-processing relationships in complex fluids to optimize their formulation, processing, and application.

The implementation of modern OSP rheology was not the result of a sudden epiphany; rather, it is based on many decades of development of custom devices. The first custom made OSP apparatus is dated back to 1966 by Simmons[6], and many efforts were made thereafter[7-10]. Those early custom-built devices suffer from many drawbacks such as alignment issues, the pumping flow effect (due to the axial movement of the bob to provide orthogonal oscillation), and limits to instrument sensitivity. In 1997, Vermant et al.[3] modified the force rebalance transducer (FRT) on a commercial separate motor-transducer rheometer, which enabled OSP measurements for fluids with a wider viscosity range than previous devices. This modification enables the normal force rebalance transducer to function as a stress-controlled rheometer, imposing an axial oscillation in addition to a measurement of the axial force. Recently, the geometries required for OSP measurements, after the methodology by Vermant, have been released for a commercial separate motor-transducer rheometer.

Since the advent of commercial OSP rheology, there is a growing interest in applying this technique for the investigation of various complex fluids. Examples include colloidal suspensions[11,12], colloidal gels[13,14] and glasses[15-17]. While the availability of the commercial instrument promotes OSP research, the complicated OSP geometry requires a deeper understanding of the measurement than other routine rheological techniques. The OSP flow cell is based on a double-wall concentric cylinder (or Couette) geometry. It features an open top and open bottom design to enable fluid to flow back and forth between the annular gaps and the reservoir. Despite the optimization made to the geometry design by the manufacturer, when undergoing OSP operation the fluid experiences an inhomogeneous flow field, geometric end effects, and residual pumping flow, all of which can introduce substantial experimental error. Our previous work[18] reported important end-effect correction procedures using Newtonian fluids for this technique. To obtain correct viscosity results, appropriate end-effect factors in both primary and orthogonal directions should be applied. In this protocol, we aim to present a detailed calibration methodology for the OSP rheological technique and provide recommendations for best practices to reduce measurement errors. The procedures delineated in this paper on OSP geometry setup, sample loading, and OSP test settings should be easily adoptable and translated for non-Newtonian fluids measurements. We advise that users utilize the calibration procedures described here to determine the end-effect correction factors for their applications prior to OSP measurements on any fluid classification (Newtonian or Non-Newtonian). We note that the calibration procedures for end factors have not been reported previously. The protocol provided in the present paper also describes step-by-step guide and tips on how to perform accurate rheological measurements in general and the technical resource on the understanding of “raw” data versus “measured” data, which may be overlooked by rheometer users.

PROTOCOL:

1. Rheometer setup

NOTE: The protocol in this section describes basic steps to run a rheology experiment (for either a separate motor-transducer rheometer or a combined motor-transducer rheometer), including preparation of the setup, installation of appropriate geometry, loading the test material, setting up the experiment procedure, specifying the geometry, and starting the test. Specific instructions and notes are provided for OSP operation. To minimize thermal gradients in the transducer, it is recommended to power the rheometer for at least 30 min prior to the operation. The rheometer software used in this protocol for instrument control and data collection are noted in the **Table of Materials**. See **Table 1** for rheometer specifications.

1.1. Before setting up the rheometer, enable the **Orthogonal Superposition** feature in the rheometer software. Install a lower platinum resistance-thermometer (PRT) on the test station for temperature measurement and an environmental control device.

NOTE: Lift the stage to maximum height for the installation process (**Figure 1a**). Install proper PRT before mounting the environmental control device. Be careful to not hit the PRT with the environmental control device during installation. Use the supplied spanner wrench to secure the environmental control device on the test station.

1.2. Install the double wall concentric cylinder geometry.

1.2.1. Assemble the inner and outer cylinders (**Figure 1b**) properly to complete the double-wall cup configuration.

NOTE: Before assembling the cup, check the O-ring condition (for cracks, swelling or other damage) on the inner cylinder and replace if needed.

1.2.2. Insert the cup into the environmental control device and align the geometry properly.

1.2.3. Press the lower geometry (cup) downward to compress the spring-loaded PRT while tightening the thumbscrew using a torque screwdriver (0.56 N m fixed).

NOTE: To check if the lower geometry is correctly installed, disable the motor power, and use a finger to spin the geometry. If the lower geometry spins freely in the environment control device, then it is installed properly and continue the next step. If it does not spin freely, remove the components from the test station in reverse order of the previous steps and then re-install the lower geometry. Verify that the temperature signal is received from the lower PRT. The rheometer should automatically recognize the temperature sensor by default; if not, select the lower PRT as the Temperature Control Sensor source in the temperature control options from the rheometer software.

1.2.4. Install the upper geometry (bob) on the transducer shaft. Tare the normal force and torque by clicking the **Tare Transducer** button in the transducer control panel from the rheometer software or using **Tare Torque** and **Tare Normal** on the Instrument tab from the instrument touch screen. A picture of the complete rheometer setup is shown in **Figure 1c**.

1.2.5. Zero the gap between the upper and lower geometries by clicking the **Zero Fixture** button in the gap control panel from either the rheometer software or from the instrument touch screen. Perform geometry mass calibration if needed.

NOTE: Check the geometry documentation supplied by the manufacturer to see if the upper tool mass value is available. If not, perform geometry mass calibration at the end of this step. Follow the on-screen instructions to perform the upper tool mass calibration. Upon completion, confirm that the correct new fixture mass is accepted.

2. Loading the test material

2.1. Lift the stage to provide enough workspace to load the test material into the cup.

2.2. Use a pipette or a spatula to load the test material into the cup. Carefully handle the test material to minimize entrainment of air into the fluid.

NOTE: For loading a low viscosity test material (e.g., less than 5 Pa s), use an adjustable volume pipette (**Figure 2a**). The minimum volume to fill the geometry can be found in the **Geometry** information under the **Experiment** panel in the rheometer software. Approximate volumes needed for the currently available OSP geometries, viz., 0.5 mm and 1.0 mm annular gap width, are 32 mL and 36 mL, respectively. For loading a higher viscosity test material (e.g., higher than 5 Pa s), use a spatula or a positive-displacement pipette (**Figure 2b**). Since precise volume control for a highly viscous liquid is difficult, fine adjustment based on the fluid volume is not recommended for loading a high viscosity liquid. In any case, it is expected to slightly underfill rather than overfill in this step. Follow the next step to ensure precise loading of material.

2.3. Lower the bob into the cup to the geometry gap set point and lifted out to determine the fluid level in the loaded geometry. The goal is to achieve a fluid contact line that is slightly (approximately 2 mm) above the lower edge of the bob upper opening.

NOTE: This process may require lengthy wait times to reach the desired fluid level because of the small annular gap width of the geometry and the relatively large volume of sample needed. The wait times mainly depend on the viscosity of the test material. For example, a highly viscous liquid takes longer to flow into the gaps between the cylinders and completely wet the bob surfaces.

2.4. Lower the upper geometry carefully into the fluid to reach the geometry gap set point of 8 mm. This process is illustrated as Step 1 in **Figure 2c**. Wait a few min while the bob is being kept at position (iii) where the gap is set to 8 mm.

NOTE: When the bob end surface contacts the fluid, reduce the downward velocity of the bob. For a high viscosity liquid or yield-stress fluid, closely monitor the normal force readings to prevent the transducer from overloading during this process.

2.5. Lift the bob vertically using the slow slew speed of the instrument to a position where the wetted fluid contact line can be visually inspected (**Figure 3**). The contact line indicates the fluid level in the geometry at the gap set point. If the line on the bob is below the upper end of the bob (lower rim of the upper opening on the bob), it indicates that the fluid height is lower than the inner cylinder height and additional test material should be added to the geometry.

2.6. Carefully lift the bob to the previous loading position to allow for enough workspace (Step 2 in **Figure 2c**) and load an additional amount of test material into the cup as needed. Slowly move the bob up or down to avoid cavitation. Add the test material carefully to prevent introducing additional air bubbles.

2.7. Lower the upper geometry into the fluid and set to the final geometry gap again. Repeat Steps 1 and 2 (**Figure 2c**) until the wetted contact line on the bob is approximately 2 mm above the lower rim of the upper bob opening as shown in **Figure 3a**. Also check that the lower rim of the upper opening on the bob is properly wetted (**Figure 3b**). Move the bob to the geometry gap set point and allow the test material to relax.

NOTE: The wait time depends on the viscosity of the standard material. For example, for a 1 Pa s liquid, a wait time of 15 min is sufficient; whereas for a 100 Pa s liquid, a much longer wait time (4 h) is needed. This process is illustrated as Step 3 in **Figure 2c**. The complete sample loading procedure is illustrated in **Figure 2**. High viscosity fluids require extended time and are difficult to load. To reduce the wait time, increasing the temperature by a few degrees may be helpful to facilitate the viscous calibration liquid to flow.

3. Running viscosity calibration measurements

NOTE: The calibration protocols provided in this paper are specific to the end-effect factors applied for the OSP technique. This does not include routine calibrations or verification checks, including torque and normal force calibrations, phase angle check, PDMS check, etc. that are recommended by individual rheometer manufacturers. Those procedures should be performed prior to the calibration protocols in the present paper. The readers should refer to the User Manual of the rheometer manufacturer for the procedures of performing routine calibrations or checks. The silicone viscosity standards used in this protocol are noted in the **Table of Materials**.

3.1. Specify the geometry

NOTE: Before setting up the experiment, make sure the correct geometry is selected in the rheometer software. For first-time use, create a new orthogonal double wall concentric cylinder geometry in the rheometer software following the steps below.

3.1.1. Add a new orthogonal double wall concentric cylinder geometry.

3.1.2. Enter the dimensions for the geometry as shown in **Table 2**.

NOTE: The numbers and their corresponding symbols are inscribed on the bob and cup. The operating gap is 8 mm for the experimental geometry used here but should be specified by the manufacturer. Therefore, the inner cylinder height is equal to the immersed height + 8 mm.

3.2. Specify the geometry constants. Fill in the fields of geometry inertia and geometry mass with proper values. Enter 1.00 for both the end-effect factor and orthogonal end-effect factor.

NOTE: The geometry inertia for the 0.5 mm and 1.0 mm gap OSP geometries specified by the manufacturer are 15.5 µN m s^2 and 10.3 µN m s^2, respectively. Make sure that the correct value for the upper geometry mass is entered. This value can be found in the geometry documentation provided by the manufacturer. Alternatively, perform geometry mass calibration under the geometry calibration tab (Protocol step 1.3) and confirm that the correct new fixture mass is applied. The default end-effect factor (c_L) is 1.065 and the orthogonal end-effect factor (c_{Lo}) is 1.04. Change both fields to 1.00. The stress constants are automatically calculated from the dimensions and end-effect factors. The strain constants are determined by the geometry dimensions only (expressions are derived here[18]). The definitions of the dimensions are described in **Table 2** and indicated in **Figure 4**. The expressions for the (primary) stress constant, K_τ, and orthogonal (linear) stress constant, $K_{\tau o}$, are:

$$K_\tau = \frac{1}{c_L} \cdot \frac{1}{2\pi h({R_2}^2+{R_3}^2)} \quad (1)$$

$$K_{\tau o} = c_{Lo} \cdot \frac{1}{2\pi h(R_2+R_3)} \quad (2)$$

4. Steady shear rate sweep tests

NOTE: Viscosity calibration measurements are performed independently in either the primary direction or the orthogonal direction to calibrate c_L or c_{Lo}. For the primary direction, steady shear viscosity is measured by performing shear rate sweep tests. For the orthogonal direction, dynamic complex viscosity is measured by performing orthogonal frequency sweep tests.

4.1. Condition the sample at 25 °C for 15 min to allow the test material to reach thermal equilibrium.

NOTE: The calibration measurements are performed at the temperature at which the certified viscosity of the standard liquid is reported, i.e., 25 °C. The readers may use a different test temperature suitable for their Newtonian standard liquids. An equilibration time or soak time, i.e., 15 min, is recommended to ensure that the environmental control device, geometries, and sample to reach thermal equilibrium.

4.2. Select the **Flow Sweep Test** under the **Experiment Procedure** in the rheometer software. Set the test temperature to 25 °C under **Environment Control**.

4.3. Specify the shear rate range from 0.01 s^{-1} to 100.0 s^{-1} with data recording at 10 points per decade logarithmically. Enable automatic steady-state determination.

NOTE: The shear rate range used here is based on the instrument torque sensitivity limits (**Table 1**) and the measuring liquid. For example, for a higher viscosity liquid (e.g., 300 Pa s), a lower shear rate range of 10^{-4} s^{-1} to 1 s^{-1} may be used, and vice versa.

4.4. Start the experiment from the rheometer software.

5. Orthogonal frequency sweep tests

5.1. Set the normal force transducer to **FRT mode** from the transducer control panel in the rheometer software.

NOTE: The default transducer setting for the normal force transducer is spring mode for this separate motor-transducer rheometer. In the OSP operation, the normal force transducer operates as a stress-controlled or a combined motor-transducer rheometer to apply axial deformation while measuring the axial force simultaneously. The normal force transducer must be set in FRT mode to perform OSP tests.

5.2. Condition the sample at 25 °C for 15 min to ensure thermal equilibration.

5.3. Select the **Orthogonal Frequency Sweep** test under the **Experiment Procedure** in the rheometer software. Set the test temperature to 25 °C.

5.4. Specify the desired normal strain and enter 0.0 s^{-1} for the shear rate in the rotational direction.

NOTE: The maximum normal strain (axial strain amplitude) is dependent on the gap width of the OSP geometry and is limited by the maximum orthogonal oscillation displacement of the rheometer, i.e., 50 µm (**Table 1**).

5.5. Specify the angular frequency range from 0.1 to 40 rad/s at 10 points per decade logarithmically.

NOTE: The angular frequency range used here is a recommended range for OSP operation based on the instrument axial frequency sensitivity limits (**Table 1**) and the consideration of gap loading conditions[18]. See the **Discussion** section for more details.

5.6. Start the experiment from the rheometer software.

6. Performing analysis

6.1. Determination of the primary end-effect factor

6.1.1. Export the steady shear rate sweep results (from Protocol step 4.4.) into an open file format such as .csv or .txt.

6.1.2. Calculate the average value of the reported viscosities over the appropriate shear rate range in a spreadsheet software.

NOTE: Only the viscosity data with corresponding torque values above the manufacture specified limits are used to calculate the average viscosity. The averaged viscosity value is defined as uncorrected primary viscosity.

6.1.3. Find the primary end-effect factor using the averaged viscosity value.

NOTE: This section is provided here to show the derivation of the relationship between the primary end effect factor and the direct viscosity output from the rheometer software. An example of the calculation of the end factor from the experimental data is demonstrated in the Representative Results section. The primary steady shear viscosity is the ratio of shear stress τ to the shear rate $\dot{\gamma}$, which is calculated from the raw signals of torque M and rotational velocity Ω via the geometry constants (K_τ and K_γ). The expression is given by:

$$\eta_{||} = \frac{\tau}{\dot{\gamma}} = \frac{K_\tau \cdot M}{K_\gamma \cdot \Omega} \qquad (3)$$

where K_τ is the primary stress constant (Equation 1) and K_γ is the primary strain constant which is solely dependent on the geometric dimensions. Therefore, substituting Equation 1 into

Equation 3, the calculated primary viscosity, or the output viscosity values from the rheometer software, is shown to be inversely proportional to the primary end-effect factor c_L (note that all other variables in Equation 3 are either geometric constants or raw measurement signals):

$$\eta_{||} \propto \frac{1}{c_L} \qquad (4)$$

Note that Equation 3 is a general expression to any rotational rheometry where the measured viscosity is calculated from the raw data, i.e., torque and velocity, via the stress and strain constants that depend on different geometry used, e.g., cone-plate, parallel plate, concentric cylinder, etc.

7. Determination of the orthogonal end-effect factor

7.1. Export the orthogonal frequency sweep results (from Protocol step 5.6.) into an open file format such as .csv or .txt.

7.2. Calculate the average value of the reported OSP complex viscosity over the appropriate angular frequency range in a spreadsheet software.

NOTE: Only the viscosity data with corresponding oscillation force values above the manufacturer specified limits are used to calculate the average viscosity. The averaged viscosity value is defined as the uncorrected orthogonal complex viscosity.

7.3. Find the orthogonal end-effect factor using the averaged complex viscosity value.

NOTE: This section is provided here to show the derivation of the relationship between the orthogonal end effect factor and the orthogonal complex viscosity output from the rheometer software. An example of the calculation of the orthogonal end factor from the experimental data is demonstrated in the Representative Results section. The orthogonal complex viscosity equals the orthogonal complex shear modulus $G_\perp^*$ divided by the orthogonal oscillatory frequency $\omega_\perp$, which can be expressed as the equation below through the oscillation force $F_\perp$, oscillation displacement $\theta_\perp$, frequency $\omega_\perp$ (all three of which are raw signals), and the geometry constants ($K_{\tau o}$ and $K_{\gamma o}$):

$$|\eta_\perp^*| = \frac{G_\perp^*}{\omega_\perp} = \frac{K_{\tau o} \cdot F_\perp}{K_{\gamma o} \cdot \theta_\perp \cdot \omega_\perp} \qquad (5)$$

where $K_{\tau o}$ is the orthogonal stress constant (Equation 2) and $K_{\gamma o}$ is the orthogonal strain constant, which is solely related to the geometric dimensions. Therefore, substituting Equation 2 into Equation 5, the calculated orthogonal complex viscosity, or the output OSP complex viscosity values from the rheometer software, is shown to be directly proportional to the orthogonal end-effect factor c_{Lo} (note that all other variables in Equation 5 are either geometric constants or raw measurement signals):

$$|\eta_{\perp}^{*}| \propto c_{Lo} \qquad (6)$$

Note that Equation 5 is a general expression for any linear motion measurements where the measured complex viscosity is calculated from the raw data, i.e., force, displacement, and frequency, via the stress and strain constants that depend on the geometry used, e.g., cone-plate, parallel plate, concentric cylinder, etc.

8. Viscosity verification check by OSP measurements

NOTE: This step is to verify if the corrections are valid using the calibrated end-effect factors obtained from the calibration experiments.

8.1. Enter the calibrated values for the end-effect factor and orthogonal end-effect factor under the geometry constants, initially these values were set equal to 1.00. The stress constants are automatically updated, and the values are as shown in **Table 3**.

8.2. Set up a same experimental procedure following the steps in the orthogonal frequency sweep tests. Enter 1.0 s^{-1} for the shear rate.

8.3. Start the experiment.

REPRESENTATIVE RESULTS:

Representative results from the viscosity calibration measurements on a 12.2 Pa s silicone viscosity standard are represented in **Figure 5** and **Figure 6**. Note that the primary end-effect factor and the orthogonal end-effect factor are both set to 1.00 for the calibration runs. **Figure 5** shows the steady shear viscosity and the torque as a function of shear rate on a double y-axis plot. The silicone liquid is a Newtonian fluid; as expected, a constant viscosity independent of the applied shear rate is obtained. The measured torque increases linearly as shear rate increases, and all of the data are above the low torque limit, 0.1 µN m, according to the manufacturer' specifications (**Table 1**). Therefore, all the viscosity data in **Figure 5** are used to calculate the average value, i.e., 14.3 Pa s (η_{uncorr}). Note that this uncorrected viscosity value is higher than the actual viscosity, i.e., 12.2 Pa s (η_{corr}), as shown by the solid line in **Figure 5**, by 17 %. According to Equation 4, the primary viscosity is inversely proportional to c_L, so the new c_L that should be applied to obtain the correct viscosity is:

$$c_{L,\text{corr}} = \frac{\eta_{\text{uncorr}}}{\eta_{\text{corr}}} \times c_{L,\text{uncorr}} \qquad (7)$$

Therefore, the correct primary end-effect factor c_L is equal to 14.3 Pa s divided by 12.2 Pa s ($c_{L,\text{uncorr}}$ = 1.00) that equals 1.17.

Figure 6 shows the results from the orthogonal frequency sweep tests at different orthogonal strain amplitudes from 0.5 % to 9.4 % for the 12.2 Pa s viscosity standard. A Newtonian response is observed, as shown by the constant orthogonal complex viscosity with varying frequency. Similarly to the primary viscosity, without correction ($c_{Lo,\mathrm{uncorr}}$ = 1), the measured orthogonal complex viscosity overestimates the actual viscosity of 12.2 Pa s (η_{corr}), as depicted by the solid line. All the viscosity data at different strains coincide with each other, indicating that the applied strains are in the linear range. The measured oscillation force plotted on the right y-axis, increases linearly with increasing frequency (Equation 5). The dashed line in **Figure 6** represents the lower limit of the axial oscillation force for the transducer, i.e., 0.001 N (**Table 1**). Only the viscosity data with corresponding orthogonal force values above this sensitivity level are used to calculate the average viscosity for correction. The averaged orthogonal complex viscosity is 15.4 Pa s (η_{uncorr}), which is 26 % higher than the actual viscosity. According to Equation 6, the orthogonal complex viscosity is proportional to c_{Lo}, so the expression for the new c_{Lo} is:

$$c_{Lo,\mathrm{corr}} = \frac{\eta_{\mathrm{corr}}}{\eta_{\mathrm{uncorr}}} \times c_{L,\mathrm{uncorr}} \qquad (8)$$

Therefore, the correct orthogonal end-effect factor c_{Lo} is equal to 12.2 Pa s divided by 15.4 Pa s ($c_{Lo,\mathrm{uncorr}}$ = 1.00) that equals 0.79.

After obtaining the calibrated values for c_L and c_{Lo}, it is recommended to run a verification test by performing an orthogonal superposition measurement under steady shear. Compared to the calibration measurements, which used only primary or oscillatory shear, both flow modes are employed simultaneously. The steady shear viscosity and orthogonal complex viscosity are measured from a single test, and the results are shown in **Figure 7**. Also plotted in the figure are the orthogonal oscillation force on the right y-axis. Only the data with values greater than the instrument force resolution are plotted. Since the correct end-effect factors are applied (**Table 3**), the measured viscosities in both directions match the accepted oil viscosity value of 12.2 Pa s. This graph can be generated by adding those outputs as plotting variables and displaying in the rheometer software for a quick check of the calibration procedure.

FIGURE AND TABLE LEGENDS:

Figure 1: Pictures of the rheometer, the OSP geometry, and the Advanced Peltier System (APS). (**a**) Rheometer test station. (**b**) Components of the orthogonal double wall concentric cylinder geometry: the outer cylinder (I), inner cylinder (II), and the center cylinder or bob (III); the PRT (IV), the torque screwdriver (V), and the spanner wrench (VI). See **Table of Materials** for the part number. The PRT, torque screwdriver, and spanner wrench are included in the APS kit. (**c**) The rheometer setup after the installation of the environmental control device and the orthogonal double wall concentric cylinder geometry for experiments. Please click here to view a larger version of this figure.

Figure 2: Detailed procedure of loading test materials. (**a**) Loading a less viscous test material using a pipette. (**b**) Loading a higher viscosity test material using a spatula. (**c**) After loading the desired amount of test materials into the cup, slowly insert the bob and decrease the gap to the geometry gap (Step 1); Lift the bob to check the fluid level by examining the wetted contact line

(Step 2); Repeat this procedure while adjusting the volume of the test material until the bob is properly wetted (Step 3). See text for details. Please click here to view a larger version of this figure.

Figure 3: Visual inspection of the wetted fluid contact line on the bob after lifting the bob out of the double wall cup. (**a**) Front view showing the contact line slightly above the upper bob end. (**b**) Side view showing the lower rim of the upper openings on the bob is properly wetted. The white dashed lines indicate the wetted fluid contact line on the bob. Please click here to view a larger version of this figure.

Figure 4: Schematic representations of the vertical and horizontal cross sections of OSP double-wall concentric cylinder geometry. (**a**) Vertical cross section in a 3D view. (**b**) Horizontal cross section in a 3D view. (**c**) 2D layout of the geometry indicating the dimensions (**Table 1**). Please click here to view a larger version of this figure.

Figure 5: Results from steady-shear rate sweep tests on a 12.2 Pa s viscosity standard. The primary steady shear viscosity (left y-axis) and torque (right y-axis) are shown as a function of shear rate. The solid line represents the actual viscosity of the silicone fluid. Please click here to view a larger version of this figure.

Figure 6: Results from orthogonal frequency sweep tests on a 12.2 Pa s viscosity standard. The orthogonal complex viscosity (left y-axis) and oscillation force (right y-axis) are shown as a function of angular frequency. The solid line represents the actual viscosity of the silicone fluid. The dashed line represents the axial oscillation force resolution limit 0.001 N. Different symbols correspond to frequency sweeps at different orthogonal strains. For the oscillation force data, from bottom to top: orthogonal strain (%) = (0.5, 0.7, 0.8, 1.1, 1.6, 2.0, 2.8, 3.9, 5.2, 7.0, and 9.4) %. Please click here to view a larger version of this figure.

Figure 7: Results from orthogonal superposition measurement on a 12.2 Pa s viscosity standard using the calibrated end-effect factors. The test is performed at a shear rate of 1.0 s^{-1} in the primary angular direction and an oscillatory shear strain of 5.2 % in the orthogonal direction. The orthogonal complex viscosity and primary viscosity (left y-axis) and oscillation force (right y-axis) are shown as a function of angular frequency. Please click here to view a larger version of this figure.

Table 1: Specifications of the rheometer and the Advanced Peltier System.

Table 2: The dimensions for the orthogonal double wall concentric cylinder used in the geometry setup as stated by the manufacturer.

Table 3: Geometry constants for the 0.5 mm OSP cell. The values of end-effect factor and orthogonal end-effect factor are obtained after calibration.

DISCUSSION:

In this protocol, we present a detailed experimental procedure for performing viscosity calibration measurements using Newtonian fluids for a commercial orthogonal superposition rheology technique with a double-wall concentric cylinder geometry. The calibration factors, i.e., the primary end-effect factor c_L and the orthogonal end-effect factor c_{Lo}, are determined independently by conducting steady shear rate sweep and orthogonal frequency sweep tests. After obtaining the end factors, a verification test is performed to check the calibration results. The verification test is an orthogonal frequency sweep test superimposed on the primary steady shear, so that the steady-shear viscosity and orthogonal complex viscosity are measured simultaneously. This contrasts with the calibration experiments where each individual test is conducted in the absence of the flow in the orthogonal direction. While this entire procedure is readily comprehensible and adoptable, there are several important steps in the protocol where users should proceed with purpose and care.

First and foremost is proper sample loading. A general rule is to keep the fluid level slightly above the lower rim of the upper opening on the bob, whether the test material is handled by a spatula or a volume adjustable pipette. Keep in mind that the loading process may require lengthy wait times to achieve the desired fluid level (**Figure 2**). Careful loading of the test material and controlling of the instrument stage are required to avoid the entrapment of air bubbles. By visual inspection of the wetted fluid contact line on the bob (**Figure 3**), the fluid height in the OSP geometry can be estimated. While the bob is in the up position, it is also important to check if the lower rim of the upper opening on the bob is completely wetted. This step is critical to maintain a fixed bob effective length, or a fixed nominal shear surface, which is helpful to reduce the bob end effects.

We recommended that users use Newtonian liquids with viscosities similar to the liquids for their application needs and perform the calibration measurements reported in this study. The example shown in the present paper is a 12.2 Pa s silicone liquid. The measurement range (i.e., shear rate and angular frequency) (**Figure 5** and **Figure 6**) used for this liquid is based on the instrument limitations (**Table 1**) and other measurement artifacts, for example, the instrument and fluid inertia. We have reported the appropriate shear rate and orthogonal frequency ranges for Newtonian standards with viscosities ranging from 0.01 Pa s to 331 Pa s in previous work[18]. Briefly, for the steady shear, the applicable shear rate range is constrained by the transducer torque limits. For the orthogonal shear, the suitable frequency window is subjected to the axial force range, gap width, and fluid properties. Specifically, measurements should be conducted within the gap loading limit that arises from shear wave propagation in viscoelastic fluids[19]. Understanding the measurement limitations and artifacts are important to avoid any misinterpretation of experimental data[20].

We define unity (1.00) as the uncorrected values for the primary end-effect factor $c_{L,\mathrm{uncorr}}$ and orthogonal end-effect factor $c_{Lo,\mathrm{uncorr}}$ to perform the viscosity calibration runs. In fact, the initial values entered for the calibration experiments do not affect the determination of the calibrated end factors. According to Equations 7 and 8, both $c_{L,\mathrm{uncorr}}$ and $c_{Lo,\mathrm{uncorr}}$ act as scale factors for the calculations of $c_{L,\mathrm{corr}}$ and $c_{Lo,\mathrm{corr}}$. To put it another way, the raw measurement signals (in Equations 3 and 5), i.e., torque M, velocity Ω, orthogonal oscillation force $F_\perp$, displacement $\theta_\perp$, and frequency $\omega_\perp$, do not depend on the end factor settings in the rheometer software. Regardless, we choose to use 1.00 in the geometry constant settings, simply for the ease of analysis, such that we can find the amount of correction needed by the viscosity outputs from the software in a straightforward manner, as well as discern whether it is overestimation or underestimation if no correction is applied. In both directions, without correction, the measured viscosity overestimates the actual viscosity, as indicated by a greater than unity value for the end-effect factor (1.17) and a less than unity value (0.79) of the orthogonal end-effect factor (**Table 2**).

The goal of the present paper is to provide visual demonstration of the experimental procedure for the calibration of end-effect factors using Newtonian viscosity standards. For detailed results and analysis of the sources of error for this commercial OSP technique, the readers should refer to our previous publication[18]. In that work, we performed computational fluid dynamics (CFD) simulations to visualize the velocity, pressure, and the shear rate fields within the entire OSP geometry. The overestimation of primary viscosity is due to a higher average shear rate in the double gap; and the overestimation of the orthogonal viscosity is attributed to the pressure forces on the bob ends in addition to a higher shear rate in the double gap. In addition, error comparisons were discussed among different instruments and between the two commercially available gap size geometries (viz., 0.5 mm and 1.0 mm). We strongly recommend that users determine the end-effect correction factors for their own instrument and geometry, because the actual corrections are material-dependent and will vary among instruments and geometries. The protocol presented in this work is critical to support the growing interest from academic and industrial users that want to apply this technique. Suitable end-effect factors should be applied to obtain correct results, otherwise the errors are appreciable.

The present calibration procedures are carried out for Newtonian fluids, which suggest that the corrections for non-Newtonian fluids could be even larger due to a more complicated flow field within the OSP geometry. As the measurement reliability for non-Newtonian fluids by OSP remains a general concern among the rheology community, future studies will focus on the quantification of end effects and other detrimental effects on the experimental error for non-Newtonian fluids. Understanding the correction related to Newtonian fluid viscosity measurements and the flow field non-idealities within the complicated OSP geometry is the first step for the application of the OSP technique. The protocol presented in this paper paves the way for future investigation on non-Newtonian fluids in order to avoid artifacts and experimental error bias for OSP research.

ACKNOWLEDGMENTS:

Ran Tao would like to thank funding from the National Institute of Standards and Technology, U.S. Department of Commerce under grant 70NANB15H112. Funding for Aaron M. Forster was provided through congressional appropriations to the National Institute of Standards and Technology.

DISCLOSURES:

The full description of the procedures used in this paper requires the identification of certain commercial products and their suppliers. The inclusion of such information should in no way be construed as indicating that such products or suppliers are endorsed by NIST or are recommended by NIST or that they are necessarily the best materials, instruments, software or suppliers for the purposes described.

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Figure 1

Figure 2

Figure 3

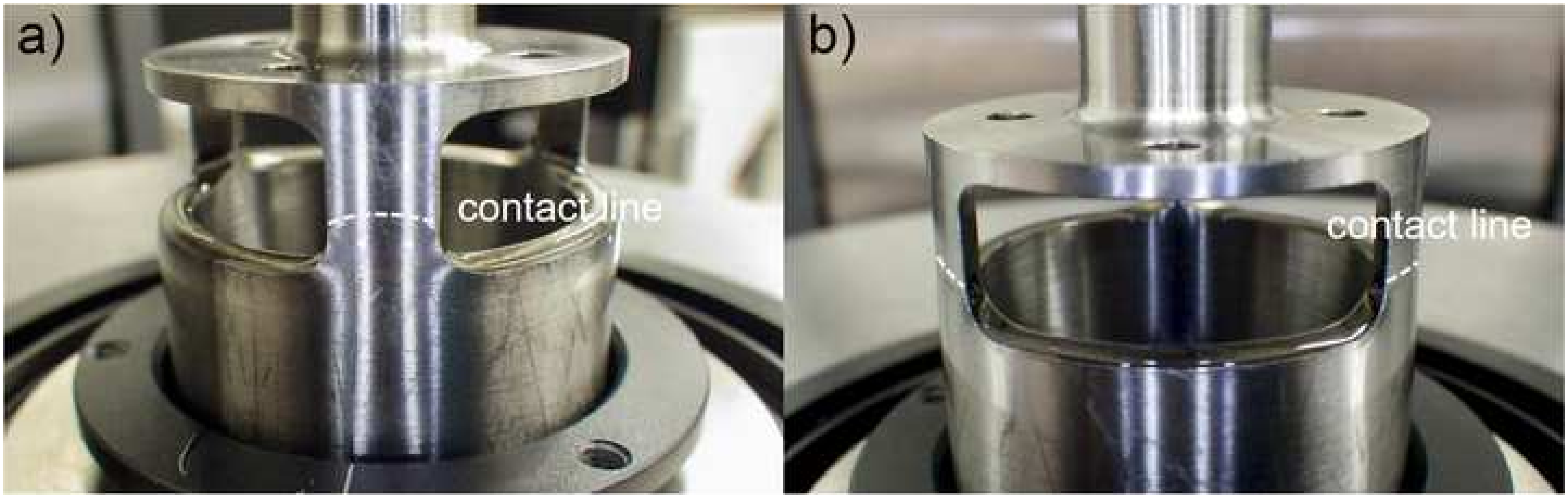
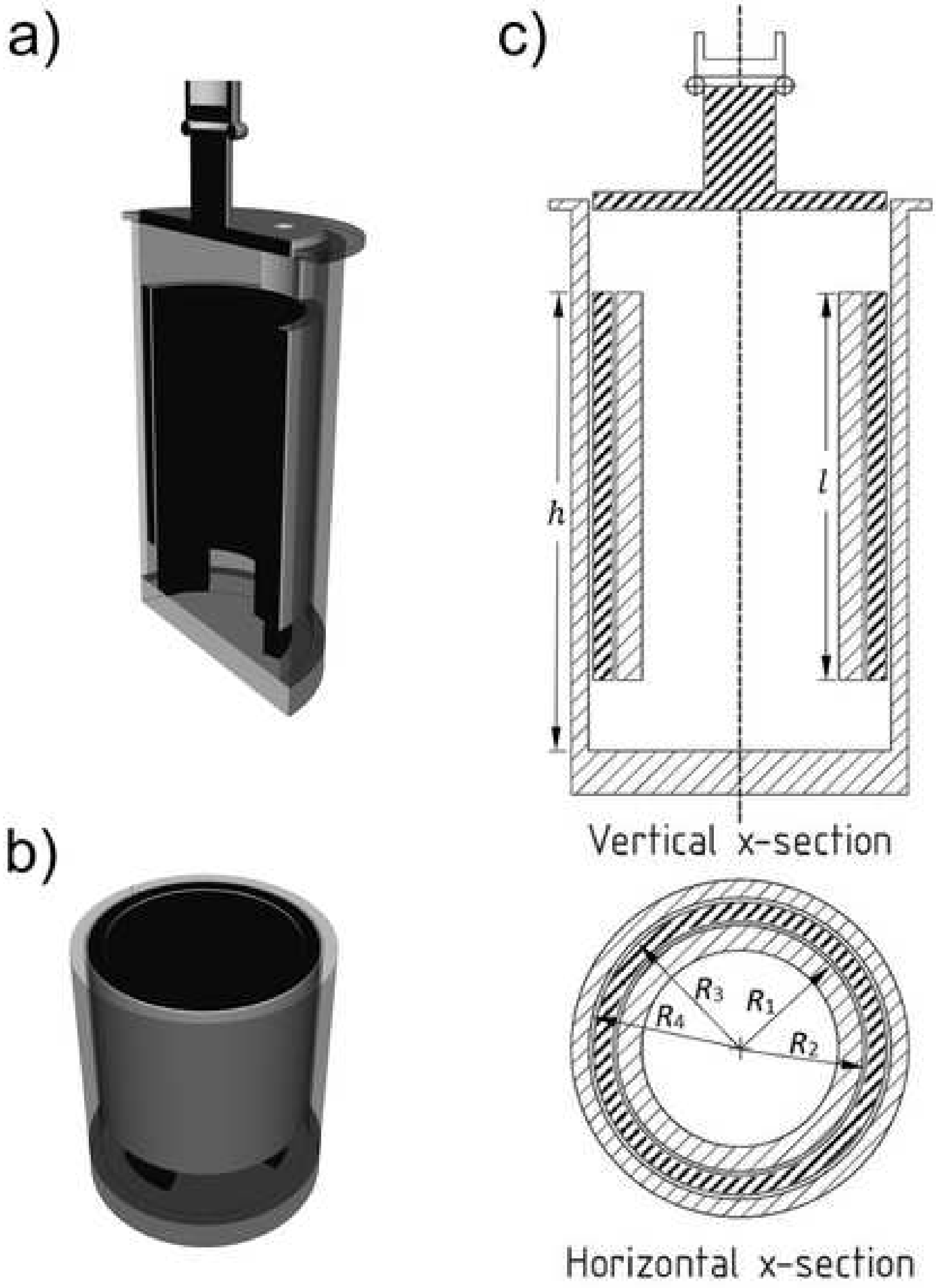
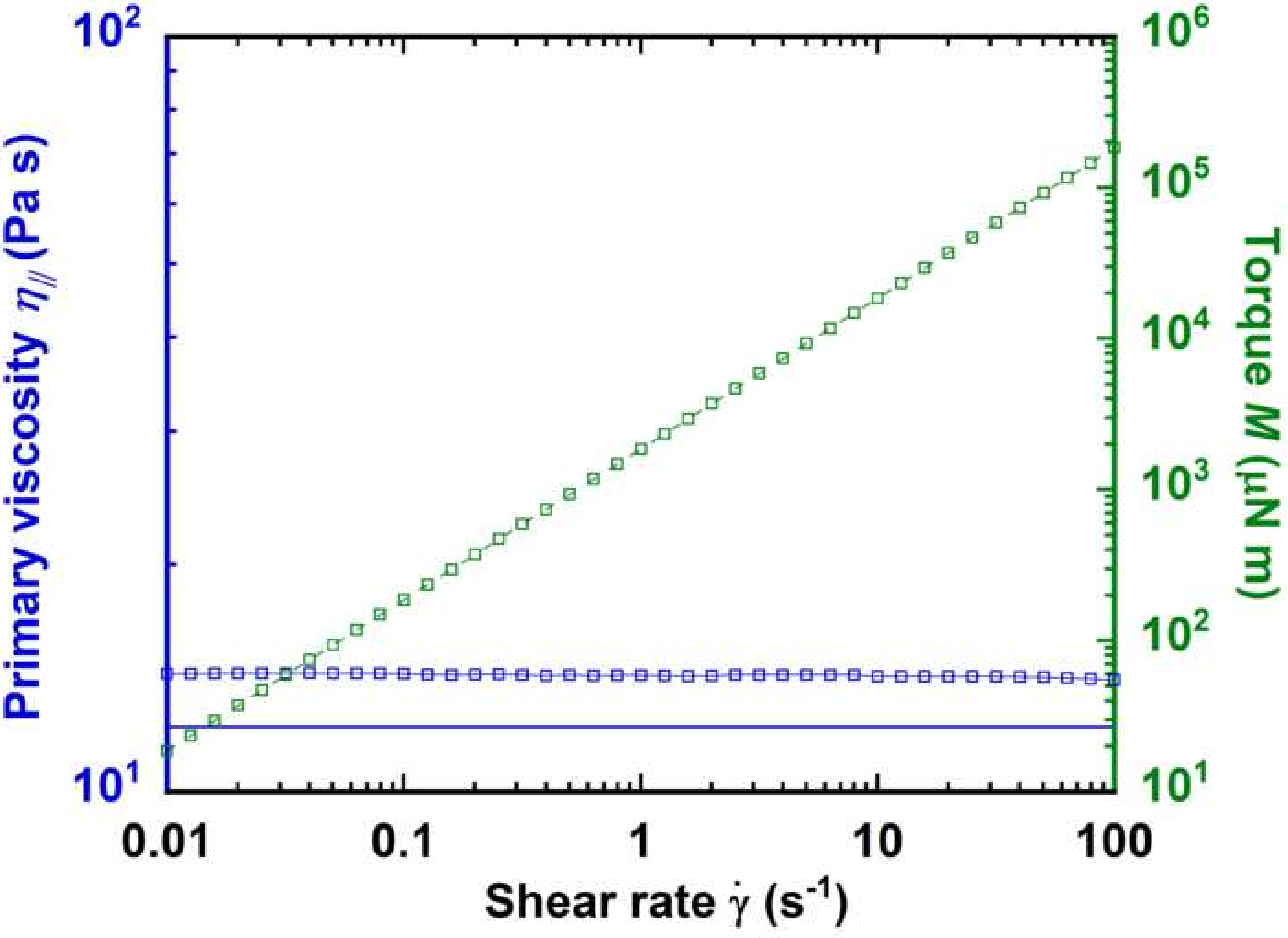
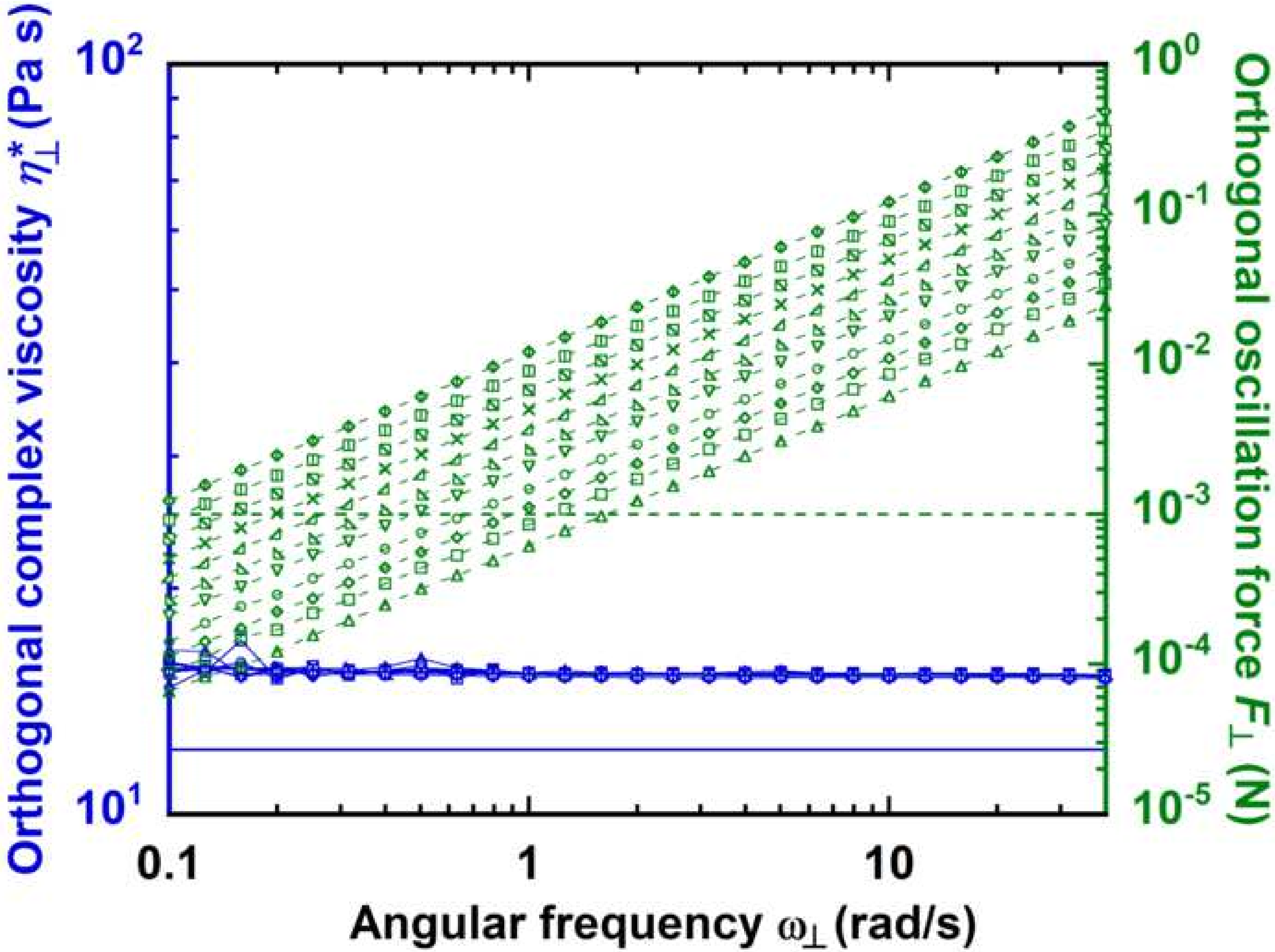
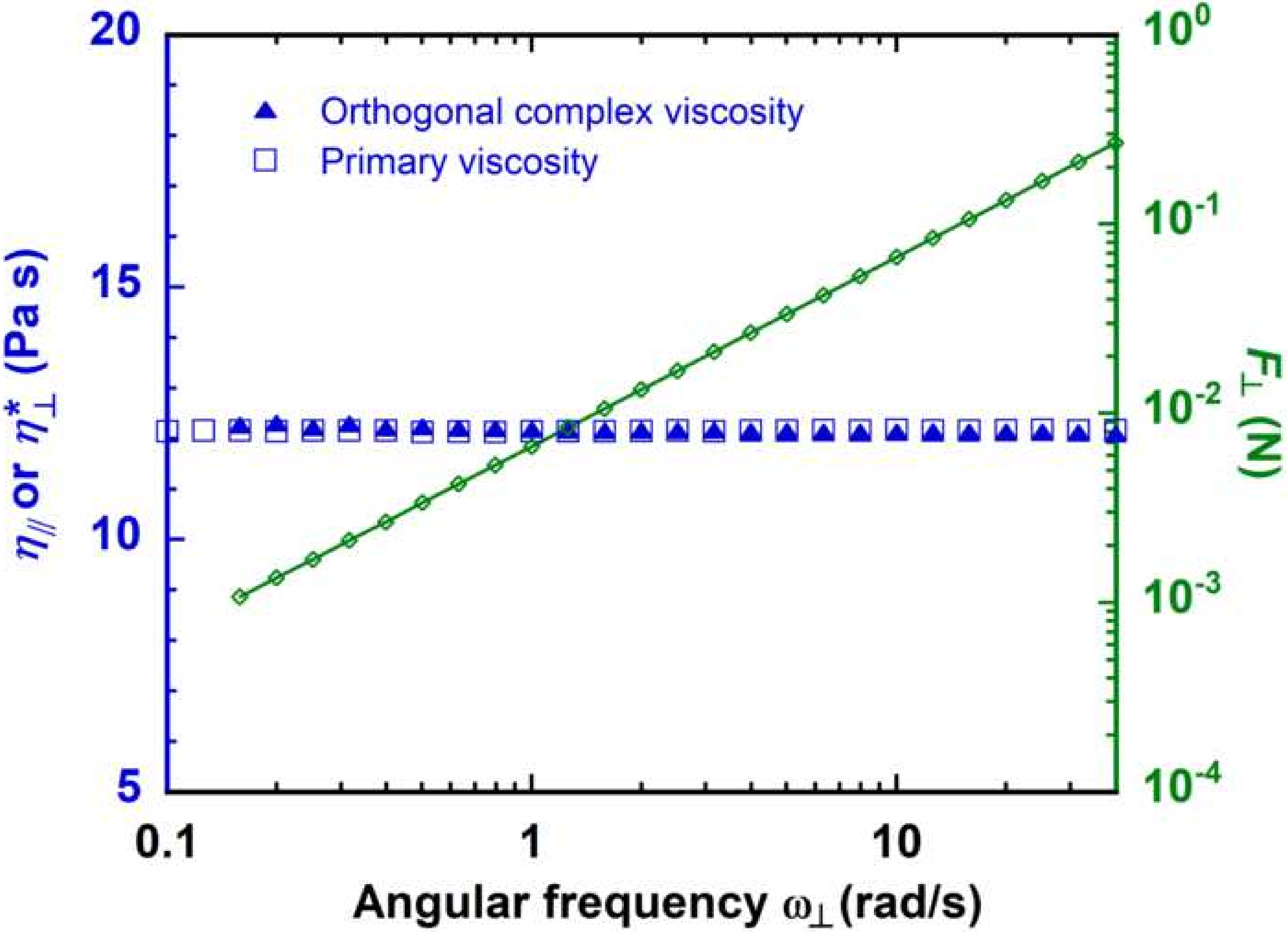


Figure 4




10^2
10^1
Primary viscosity $\eta_{//}$ (Pa s)
10^6
10^5
10^4
10^3
10^2
10^1
Torque M (μN m)
0.01
0.1
1
10
100
Shear rate $\dot{\gamma}$ (s^{-1})


10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
0.1
1
10
Orthogonal complex viscosity $\eta_\perp^*$ (Pa s)
Orthogonal oscillation force $F_\perp$ (N)
Angular frequency $\omega_\perp$ (rad/s)


▲ Orthogonal complex viscosity
□ Primary viscosity
20
15
10
5
$\eta_{//}$ or $\eta^*_\perp$ (Pa s)
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
$F_\perp$ (N)
0.1
1
10
Angular frequency $\omega_\perp$ (rad/s)

Parameter description	Specifications
Minimum transducer torque in steady shear	0.1 µN m
Maximum transducer torque	200 mN m
Torque resolution	1 nN m
Normal/axial force range	0.001 N to 20 N
Angular velocity range	10^{-6} rad s^{-1} to 300 rad s^{-1}
Minimum force in oscillation (OSP mode)	0.001 N
Minimum displacement in oscillation (OSP mode)	0.5 µm
Maximum displacement in oscillation (OSP mode)	50 µm
Displacement resolution (OSP mode)	10 nm
Axial frequency range (OSP mode)	6.28×10^{-5} rad s^{-1} to 100 rad s^{-1}
APS temperature range	−10 °C to 150 °C

Parameters in geometry setup	Inscribed abbreviation	Dimension (mm)	Symbol in stress constants
Inside cup diameter	CID	27.733	$2R_1$
Inside bob diameter	ID	28.578	$2R_2$
Outside bob diameter	OD	32.997	$2R_3$
Outside cup diameter	COD	33.996	$2R_4$
Immersed height (cup height)	CH	43.651	h
Inner cylinder height		51.651	l

End-effect Factor	1.17
Orthogonal End-effect Factor	0.79
Stress Constant	6541.69 Pa N^{-1} m^{-1}
Strain Constant	33.4326 rad^{-1}
Stress Constant (Linear)	93.5575 Pa N^{-1}
Strain Constant (Linear)	2136.55 m^{-1}

Name of Material/Equipment	Company	Catalog Number	Comments/Description
Advanced Peltier System	TA Instruments	402500.901	Enviromental control device
ARES-G2 Rheometer	TA Instruments	401000.501	Rheometer
Brookfield Silicone Fluid, 12500cP	AMTEK Brookfield	12500 cps	Viscosity standard liquid
OSP Slotted Bob, 33 mm	TA Instruments	402796.902	Bob, upper geometry
OSP Slotted Double Gap Cup, 34 mm	TA Instruments	402782.901	Double wall cup, lower geometry
Pipette (1 – 10 mL)	Eppendorf	3120000089	To load test materials
Pipette (100 – 1,000 µL)	Eppendorf	3123000063	To load test materials
Pipette Tips (0.5 – 10 mL)	Eppendorf	022492098	To load test materials
Pipette Tips (50 – 1,000 µL)	Eppendorf	022491555	To load test materials
Spatula	VWR	82027-532	To load test materials
TRIOS	TA Instruments	v4.3.1.39215	Rheometer software

Response to Review

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

Author reply: We appreciate the editor's comments to improve the quality of the paper. The manuscript was carefully checked and a few grammatical errors were caught and corrected. All abbreviations were defined at first use.

2. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.

Author reply: We modified reference numbers format according to the editor's suggestion.

3. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.
O we
Author reply: We changed the numbered list of the Protocol to the required format.

4. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Author reply: We thank the editor for the suggestions. We have added more specific details to the protocol steps where appropriate. For example, see responses to comments #5 and #6 below. In general, the action descriptions in each numbered step provide adequate information for a rheometer user to execute the protocol. There are instances where we felt increasing the level of specific detail would violate NIST's neutral position or create confusion for users rheometers from different manufacturers.

We thank the editor for indulging our justification for this position with respect to providing "every" action item in the manuscript. We have strived to accurately describe actions in a way that is rheologically accurate, as well as to eliminate bias toward a particular manufacturer's software. NIST is an independent measurement laboratory, a government agency that must hold a neutral position to any vendor and manufacturer, and we do not endorse any commercial products and their suppliers. The calibration protocol reported in this paper aims to provide recommendations for best practices to use the OSP technique, avoid procedural mistakes, and minimize experimental error, which is expected to benefit the entire rheology community. Before submission, our manuscript has gone through reviews by our internal Editorial Review Board and Legal team. Upon their guidance, we refrain from writing a "Standard Operating Procedure" style methods paper for a specific rheometer or citing the User Manual or Technical Note by a specific rheometer manufacturer, in accordance with NIST's position. Nevertheless, we are able to write the Protocol section with step-by-step instructions and detailed tips for those operations, which should be easily followed by rheologists using a different instrument and software. We appreciate the editor's understanding of our position.

In closing, as the editor pointed out that the numerical values for experimental settings are important, we strongly agree. Those specifics have been included in the protocol steps to allow the readers to replicate the tests. In addition to the short notes provided in the Protocol, we also emphasized several important steps where users should proceed with care, later in the Discussion section. With those detailed descriptions, we expect the entire procedure to be readily comprehensible and adoptable.

5. 2d: How do you tare the normal force and torque?

Author reply: The following text was added to Protocol 1.2.4 (lines 154 to 156) to explain how to tare the normal force and torque.

> "This can be achieved by either using the Tare Transducer button in the transducer control panel from the rheometer software or using Tare Torque and Tare Normal on the Instrument tab from the instrument touch screen."

6. 3: How do you zero the gap between upper and lower geometries?

Author reply: The following text was added to Protocol 1.3 (lines 159 to 161) to describe how to zero the gap between geometries.

> "This can be achieved by clicking the Zero Fixture button in the gap control panel from either the rheometer software or from the instrument touch screen."

7. Note after II-2, how much of the higher viscosity material do you use?

Author reply: It is difficult to precisely control the volume of a high viscosity fluid. We recommend users start with the approximate volume of the geometry, specified by the manufacturer, to load the high viscosity fluid. This is followed by using the wetting line of the bob to adjust the final volume in the geometry. The following changes were made to the Note after Protocol 2.2 (lines 174 to 183).

> "NOTE: For loading a low viscosity test material (e.g., less than 5 Pa s), use an adjustable volume pipette (**Figure 2a**). The minimum volume to fill the geometry can be found in the Geometry information under the Experiment panel in the rheometer software. Approximate volumes needed for the currently available OSP geometries, viz., 0.5 mm and 1.0 mm annular gap width, are 32 ml and 36 ml, respectively. For loading a higher viscosity test material (e.g., higher than 5 Pa s), use a spatula or a positive-displacement pipette (**Figure 2b**). Since precise volume control for a highly viscous liquid is difficult, fine adjustment based on the fluid volume is not recommended for loading a high viscosity liquid. In any case, it is expected to slightly underfill rather than overfill in this step. Follow the next step to ensure precise loading of material."

8. Note after II-3c, how do you determine if the temperature effect on the material property is fully recoverable?

Author reply: We thank the editor for the question. The calibration protocol was performed using Newtonian fluids that exhibit a temperature dependent viscosity. As long as the temperature is controlled to within the range specified for the viscosity standard, the fluid viscosity is reproducible, and the fluid is not expected to degrade within the operating temperature of the instrument. There is no need to discuss temperature-dependent properties of other test materials in the paper. The following text was removed from Note after Protocol 2.3.3.

> “This approach can be used to load other test materials as long as the temperature effect on the material property is fully recoverable.”

9. First note in III, please cite references for the routine calibrations and checks.

Author reply: In compliance with NIST’s guidance, we must refrain from citing the User Manual or Technical Note by a specific manufacturer. Please see the response to your comment #4 above. We added the following sentence to the Note after Protocol 3 (lines 238 to 240) to direct the readers to the proper reference.

> “The readers should refer to the User Manual of the rheometer manufacturer for the procedures of performing routine calibrations or checks.”

10. After revising the protocol (renumbering and adding some details), please highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Author reply: We have made those changes.

11. Please remove the embedded figures and tables from the manuscript. All figures should be uploaded separately to your Editorial Manager account. Each figure must be accompanied by a title and a description after the Representative Results of the manuscript text. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.

Author reply: We have made those changes.

12. As we are a methods journal, please add limitations of the technique to the Discussion.

Author reply: We thank the editor for this suggestion. The limitations of the technique were initially discussed in the last paragraph of the Discussion, and were modified for clarification (lines 599 to 615).

> “We strongly recommend that users determine the end-effect correction factors for their own instrument and geometry, because the actual corrections are material-dependent and will vary among instruments and geometries. The protocol presented in this work is critical to support the growing interest from academic and industrial users that want to apply this technique. Suitable end-effect factors should be applied to obtain accurate results, otherwise the errors are appreciable.

> The present calibration procedures are carried out for Newtonian fluids, which suggest that the corrections for non-Newtonian fluids could be even larger due to a more complicated flow field within the OSP geometry. As the measurement reliability for non-Newtonian fluids by OSP remains a general concern among the rheology community, future studies will focus on the quantification of end effects and other detrimental effects on the experimental error for non-Newtonian fluids. Understanding the correction related to Newtonian fluid viscosity measurements and the flow field non-idealities within the complicated OSP geometry is the first step for the application of the OSP technique. The protocol presented in this paper paves the way for future investigation on non-Newtonian fluids in order to avoid artifacts and experimental error bias for OSP research."

13. Please sort the Materials Table alphabetically by the name of the material.

Author reply: The Materials Table was revised.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Experimental errors due to sample preparation, calibration, or geometry uses are critical in rheology measurements. The authors report detailed orthogonal superposition rheology technique that will contribute insights to the field. The manuscript was well written and can be published as is, or with minor revisions as noted:

1. Page 8, lines 264- 268: It is not necessary at 25 C, 15 minutes, or that shear rate range. The authors are suggested to comment on the temperature, time, and shear rate setup based on the measuring samples.

Author reply: We first thank the reviewer for the positive feedback and helpful suggestions to improve the completeness of the paper. The following text was added to better explain the temperature and equilibration time recommendations (lines 290 to 294).

> "NOTE: The calibration measurements are performed at the temperature at which the certified viscosity of the standard liquid is reported, i.e., 25 °C. The readers may use a different test temperature suitable for their Newtonian standard liquids. An equilibration time or soak time, i.e., 15 min, is recommended to ensure that the environmental control device, geometries, and sample reach thermal equilibrium."

For the shear rate range, we would like to bring it to the reviewer's attention that those aspects were initially discussed in the third paragraph of the Discussion in the previous version of the manuscript:

"The measurement range (i.e., shear rate and angular frequency) (**Figure 5** and **Figure 6**) used for this liquid is based on the instrument limitations (**Table 1**) and other measurement artifacts, for example, the instrument and fluid inertia. We have reported the appropriate shear rate and orthogonal frequency ranges for Newtonian standards with viscosities ranging from 0.01 Pa s to 331 Pa s in previous work[18]. Briefly, for the steady shear, the applicable shear rate range is constrained by the transducer torque limits. For the orthogonal shear, the suitable frequency window is subjected to the axial force range, gap width, and fluid properties. Specifically, measurements should be conducted within the gap loading limit that arises from shear wave propagation in viscoelastic fluids[19]. Understanding the measurement limitations and artifacts are important to avoid any misinterpretation of experimental data[20]."

We also added a Note after Protocol 3.2.3 for better clarification (lines 302 to 305).

> "NOTE: The shear rate range used here is based on the instrument torque sensitivity limits (**Table 1**) and the measuring liquid. For example, for a higher viscosity liquid (e.g., 300 Pa s), a lower shear rate range of 10^{-4} s^{-1} to 1 s^{-1} may be used, and vice versa."

2. Page 8, line 291: Similarly, selection of angular frequency range should be discussed. Is there any reason to select 0.1- 40 rad/s? Also, please specify the measurement is from 40 to 0.1 rad/s or from 0.1 to 40 rad/s?

Author reply: We thank the reviewer for the questions. The selection of angular frequency range was initially discussed in the Discussion in the previous version of the manuscript. Please see the response to the previous comment #1 above. In addition, the following text was added to Protocol 3.3.5 for clarification (lines 331 to 333).

> "NOTE: The angular frequency range used here is a recommended range for OSP operation based on the instrument axial frequency sensitivity limits (**Table 1**) and the consideration of gap loading conditions[18]. See the Discussion section for more details."

3. Page 14, line 460 (Figure 3): It is difficult to observe the contact line. Should you mark or use a dotted line for the observation?

Author reply: We thank the reviewer for this observation. The figure was modified to highlight the contact line.

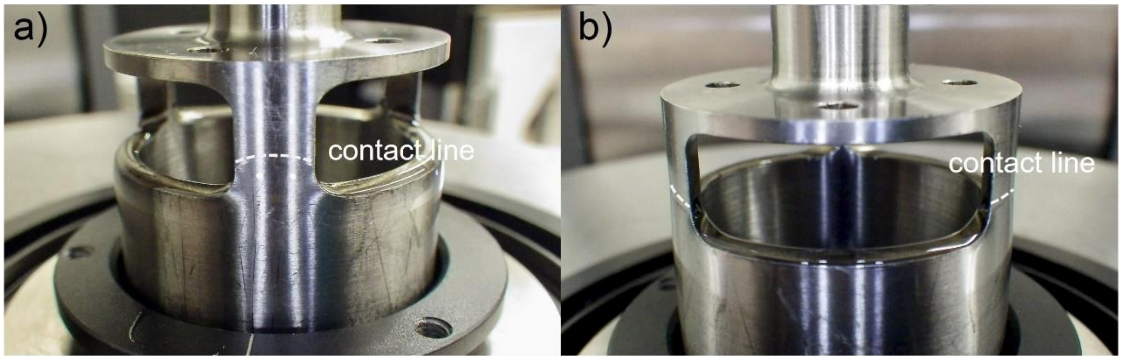


Figure 3: Visual inspection of the wetted fluid contact line on the bob after lifting the bob out of the double wall cup. (a) Front view showing the contact line slightly above the upper bob end. (b) Side view showing the lower rim of the upper openings on the bob is properly wetted. The white dashed lines indicate the wetted fluid contact line on the bob. Please click here to view a larger version of this figure.

4. Some additional comments on the sample types and temperature range capacity for the measurements are suggested.

Author reply: We thank the reviewer for the suggestions. The following text was added to address the measurement fluid types (Newtonian vs non-Newtonian) in the Discussion (lines 606 to 608). The temperature range capacity of the environmental control device is included in Table 1.

> “The present calibration procedures are carried out for Newtonian fluids, which suggest that the corrections for non-Newtonian fluids could be even larger due to a more complicated flow field within the OSP geometry.”

Reviewer #2:

Manuscript Summary:

This work describes a protocol for the calibration of the geometry-correction factors for orthogonal superposition (OSP) rheology for Newtonian fluids. Orthogonal superposition is a technique that is of significant interest for rheological measurement because it can provide easily interpretable data of fluids far from their mechanical equilibrium point, i.e. when material properties often have non-linear dependencies. The technique has had notable but relatively limited usage since its initial development, but is gradually becoming more widely adopted, especially with the advent of a commercially available system.

From my own experience working with this system, the protocol described here is well presented and would be very helpful in generating reproducible, high quality rheological data. I recommend acceptance of this work to JoVE. Below are several points that the authors may wish to consider that I believe could further clarify and improve the manuscript's impact.

Major Concerns:

My only significant concern regarding this protocol is the limitation to Newtonian fluids. Measurement of Newtonian fluids is not generally an area in which a typical researcher would apply the OSP technique and it is not necessarily the case that calibrating the OSP for a Newtonian standard would provide the correct geometric corrections for a non-Newtonian fluid. However, the authors correctly state in their last paragraph that understanding and measuring the Newtonian correction is the necessary first step in proper application of this technique. Furthermore, the setup and loading steps would likely be identical for any sample—including non-Newtonian ones—and thus still have significant value. The authors might wish to point out these aspects earlier in the manuscript.

Author reply: We first thank the reviewer for the positive feedback and helpful suggestions to improve the quality and impact of the paper. We completely agree with reviewer, and we recognize the value of the OSP technique to study non-Newtonian fluids. The procedures delineated in this paper on sample loading and experimental setup for OSP experiments should be easily adoptable and translated for non-Newtonian fluids measurements. Therefore, it is worthwhile to highlight these points earlier in the manuscript. The following text was added to the last paragraph of the Introduction (lines 96 to 98).

> “The procedures delineated in this paper on OSP geometry setup, sample loading, and OSP test settings should be easily adoptable and translated for non-Newtonian fluids measurements.”

Minor Concerns:

- In the abstract and introduction, the authors mention "non-linear flow", which I believe refers to flows that result in material properties with non-linear dependencies. Though a typical rheometer-user would likely understand what is meant by a "non-linear flow", it would be helpful to more precisely define this term and how OSP achieves it.

Author reply: We thank the reviewer for this comment. The reviewer's point is correct. We have revised the following text in the Introduction for better clarification (lines 54 to 64).

> "Unlocking this structure-property loop via non-linear viscoelastic behavior of complex fluids in response to flow and deformation remains a challenging task for experimental rheologists.
>
> Orthogonal superposition (OSP) rheology[3] is a robust technique to address this measurement challenge. In this technique, a small amplitude oscillatory shear flow is superimposed orthogonally to a unidirectional primary steady-shear flow, which enables the simultaneous measurement of a viscoelastic relaxation spectrum under the imposed primary shear flow. To be more specific, the small oscillatory shear perturbation can be analyzed using theories in linear viscoelasticity[4], while the non-linear flow condition is achieved by the primary steady-shear flow. As the two flow fields are orthogonal and thus not coupled, the perturbation spectra can be directly related to the variation of the microstructure under the primary non-linear flow[5]."

- Similarly, references are made to the "pumping flow" problem in the introduction, but I think a user who is new to OSP would not be familiar with the term. Instead I recommend considering something like, "unintended secondary flows"?

Author reply: We thank the reviewer for the suggestion. The following text was modified to define the pumping flow effect (lines 72 to 74).

> "Those early custom-built devices suffer from many drawbacks such as alignment issues, the pumping flow effect (due to the axial movement of the bob to provide orthogonal oscillation), and limits to instrument sensitivity."

- In part 1 of the protocol, it would be helpful to include an image of the short PRT. In 2c, for consistency, the "spring-loaded PRT" should be referred to rather than a thermometer.

Author reply: We thank the reviewer for the helpful suggestions. The image of the short PRT has been included in the new Figure 1b. The text "thermometer" was changed to "spring-loaded PRT" to reflect the correct description (line 140).

Figure 1: Pictures of the rheometer, the OSP geometry, and the Advanced Peltier System (APS). (a) ARES-G2 test station. (b) Components of the orthogonal double wall concentric cylinder geometry: the outer cylinder (I), inner cylinder (II), and the inner cylinder (III); the PRT (IV), the torque screwdriver (V), and the spanner wrench (VI). See Table of Materials for the part number. The PRT, torque screwdriver, and spanner wrench are included in the APS kit. (c) The rheometer setup after the installation of the environmental control device and the orthogonal double wall concentric cylinder geometry for experiments. Please click here to view a larger version of this figure.

- I recommend that after 2c, an additional step or note be added to confirm that signal from the PRT is being received. Though I'm unsure if it was a quirk of our particular installation, it was common for the rheometer to not recognize that the PRT was connected.

Author reply: We thank the reviewer for the consideration. The following text was added to the Note after Protocol 1.2.3 (lines 147 to 151).

> “Verify that the temperature signal is received from the lower PRT. The rheometer should automatically recognize the temperature sensor by default; if not, select the lower PRT as the Temperature Control Sensor source in the temperature control options from the rheometer software.”

- In part 2, a positive-displacement pipette could also be suggested for handling very viscous materials.

Author reply: We thank the reviewer for the suggestion. We have modified the Note after Protocol 2.2 (lines 178 to 180).

> “For loading a higher viscosity test material (e.g., higher than 5 Pa s), use a spatula or a positive-displacement pipette (**Figure 2b**).”

- In part 2, step 3, It would be helpful to quote some observed/recommended wait times for the highly viscous liquids. Are the few minutes specified in a) and the 15 minutes in c) for highly viscous materials or even for low viscosity ones?

Author reply: We thank the reviewer for the questions. The following text was added to Protocol 2.3.3 to describe the observed wait times (lines 220 to 222).

> “The wait time depends on the viscosity of the standard material. For example, for a 1 Pa s liquid, a wait time of 15 min is sufficient; whereas for a 100 Pa s liquid, a much longer wait time (4 hours) is needed.”

- In line 183, there should be a hyphen between "yield" and "stress" in yield-stress fluid

Author reply: We thank the reviewer for the meticulous suggestion. The suggested change was made.

- The authors appropriately cite their own related work for discussion on the analysis of sources of error, however I think it would helpful to experimentalists carrying out this protocol to provide some intuitive explanation for why they might expect the end-effect corrections to be greater than or less than unity. Along these same lines, a range of expected or observed values of the end-effect factor would be helpful as a quick check that the calibration was performed correctly.

Author reply: We thank the reviewer for the helpful suggestions. The following text was added to address those aspects in the Discussion (lines 586 to 601).

> “…In both directions, without correction, the measured viscosity overestimates the actual viscosity, as indicated by a greater than unity value for the end-effect factor (1.17) and a less than unity value (0.79) of the orthogonal end-effect factor (**Table 2**)…
>
> …The overestimation of primary viscosity is due to a higher average shear rate in the double gap; and the overestimation of the orthogonal viscosity is attributed to the pressure forces on the bob ends in addition to the higher shear rate in the double gap…
>
> … We strongly recommend that users determine the end-effect correction factors for their own instrument and geometry, because the actual corrections are material-dependent and will vary among instruments and geometries.”