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FEASIBILITY OF RHEOLOGICAL TESTING ON SMALL VITREOUS VOLUMES

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ABSTRACT

Posterior Vitreous Detachment (PVD) is a naturally occurring condition in the eye where the vitreous detaches from the retina with age. The careful balance between weakening interfacial adhesion and vitreous liquefaction results in a relatively uncomplicated occurrence. However, imbalance between these factors can lead to complications and vision loss. Understanding the symbiotic relationship between molecular biology and mechanics in PVD can help elucidate causative factors of this imbalance, and ultimately lead to innovative methods for detection, prevention, and treatment. Genetic mouse models are an ideal starting point to identify the effects of protein changes to liquefaction of the vitreous. However, the small volume of the mouse vitreous makes it challenging to quantify changes in viscosity and other mechanical parameters. In this thesis, we evaluate the feasibility of using parallel plate rheometry on small volumes, similar to mouse vitreous. Strain sweep tests, stepped flow tests, and frequency sweeps were performed on polystyrene-toluene (PST), a viscoelastic material. All tests were performed at three gap heights (50µm, 400µm, and 800µm) resulting in 3 material volumes. The effects of sandpaper on the plates for better gripping was also evaluated. It was found that the accuracy of testing small volumes (4.4µL) was strongly dependent on accurate tolerances of the contact surfaces. Sandpaper did not affect the results with large volumes, but adversely affected data with a 50µm gap height. These data suggest that parallel plate rheology is possible on small volumes at a gap height of 50µm, but care needs to be taken to accurately create parallel surfaces before testing.

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INTRODUCTION

Posterior vitreous detachment (PVD) is a common condition where the vitreous naturally shifts with age from a gel-like substance to contain pockets of fluid. At the same time, the interface between the vitreous and retina weakens, allowing for the seamless contraction and separation of the vitreous from the retina. Most people experience PVD with little or no problems. However, early vitreous liquefaction or delayed weakening of vitreoretinal adhesion creates a partial PVD which results in traction on the retina and increases risk of retinal disruption and vision loss. A careful understanding of the mechanisms and timing of vitreous liquefaction and adhesion weakening with age can elucidate prevention and treatment strategies for partial PVD.

It is suspected that collagen IX contributes to the separation of collagen fibers in the vitreous. Specifically, it is hypothesized that a reduction in this collagen protein results in vitreal collagen clustering and formation of liquefied pockets, which would alter the material properties of the vitreous. As a first step to testing this hypothesis, we plan to quantify the material properties of mouse vitreous from knockout mice where the gene encoding for collagen IX has been suppressed, and then compare data to wild-type controls. Vitreous material testing has historically been performed with parallel plate rheology [1][2][3][4]. However, this form of testing on mouse vitreous is likely to be extremely challenging giving that a mouse vitreous is only around 4.4μL [5]. This suggests that parallel plate rheometry will require a 50μm gap height with a 10mm plate. Rheometer testing on this small scale of volume and gap has not been done and will require precise calibration of the rheometer and high tolerance design of the cleats and flat plates. The objective of this thesis was to design appropriate parallel plates and validate a rheological test to quantify the viscosity and dynamic moduli of small volumes. A polystyrene-toluene (PST) mixture was selected for the study because it has known viscoelastic material properties that are similar to those of the vitreous.

METHODS

Parallel Plate Geometries

Parallel surfaces were originally constructed from PLA using FDM 3D printing. The top piece was 10mm in diameter and attached to a modular rheometer attachment that used two set screws to hold the top in place as seen in Figure 1. A 10mm diameter was chosen because larger diameter plates would require gap heights less than 50μm. Preliminary evaluation on gap heights less 50μm showed significant noise in the measurements.

Figure 1. 10mm diameter FDM PLA rheometer top plate

After many tests, it was noted that the data began to look notably different at small gap heights. Further the parallel surface had visible erosion as a result of the prolonged exposure to toluene. This effect seemed only noticeable for the 50μm gap height tests because of the higher sensitivity to small variations in the gap height. Therefore, the top parallel surface was replaced with a custom aluminum post as seen in Figure 2. This would tighten the tolerances of the part and eliminate the effects of toluene on the geometry.

Figure 2. 10mm diameter aluminum rheometer top

The part was manufactured on a manual lathe and the contact surface was polished with Mothers® Mag and Aluminum Polish to further tighten the tolerances of the contact surface.

The bottom plate was 3D printed out of PLA using FDM 3D printing. The 3D printed PLA piece was kept for the duration of the testing. Originally, the bottom surface was chosen to be larger than 10mm in diameter because it was stationary and was unlikely to have much influence on the data (Figure 3).

Figure 3. Smooth FDM PLA rheometer bottom plate

However, we noticed unusually low viscosities with larger gap heights and concluded it was from the bottom plate being too large. Upon inspection, the fill shape of the PST was not correct due to the larger diameter bottom plate (Figure 4). Therefore, we reprinted the bottom surface with a 10mm diameter to match the top plate.

Since the bottom plate was still subject to erosion from the toluene, care was taken to replace the bottom plate every 10 tests. To tighten the tolerance of the bottom plate the part was 3D printed with the contact surface facing the bed.

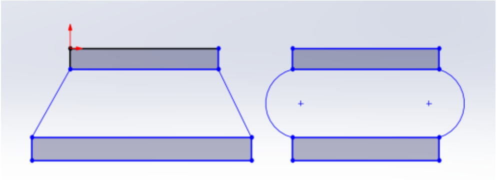


Figure 4. The shape of the fluid between the plates before the matching diameter plates (left) and after (right)

Sandpaper Cleats

Sandpaper or physical cleats are often used on the surface of parallel plates to improve gripping of the sample [6]. This is specifically important when testing vitreous, which is highly lubricated. We evaluated tests with and without sandpaper to identify their effects on the results. The sandpaper chosen was P1200 grit, which had an approximate grit size of 15.3µm in diameter [7]. The sandpaper chosen had an adhesive backing to allow sequential testing without the use of common adhesives that leave residues or require dry time. When used, the sandpaper was applied to both the top and bottom plates to avoid a boundary layer forming on either side of the contact area. About midway through testing, we noticed higher viscosities at 50µm gap heights and therefore also evaluated a small grit sandpaper, P2400. The diameter of the grains at this grit size is approximately 8.8µm. This change reduced the diameter of the sandpaper grit from 61.2% of the gap height (30.6% for each side of the gap), to around 35.2% of the gap height. After the change in sandpaper grit the viscosity was much closer to that expected at the other gap heights, but was not fully corrected until the top plate was changed to tighten its tolerances.

Rheological Testing

Before testing could begin, a PST solution needed to be created. This mixture was created by mixing 0.6g of polystyrene per 1mL of toluene [8]. The mixture was continually stirred for 2 hours before being used in any testing. Individual samples were removed from the mixture immediately before the tests were performed. The mixture was continually stirred until all tests involving that mixture were completed. To evaluate the mechanical response of the PST with different volumes, we performed a strain sweep, a stepped flow test, and a frequency sweep. The strain sweep was used to identify the linear viscoelastic region of the PST. The strain from the viscoelastic region would be used for the frequency sweep test. The stepped flow test evaluated the viscosity as a function of shear strain rate, and is commonly presented for PST in the literature. This allowed comparison to existing values. The frequency sweep test is the most common used for vitreous mechanical testing and was the final comparison for the different volumes. It also was the test used to identify whether a gap correction factor was needed when using the sandpaper. A gap correction factor has typically only been used with cleated parallel plate geometries due to alteration in the boundary layers [6]. Prior to this experimentation it was unknown if a gap correction factor would be needed with sandpaper. All tests were performed on an AR-G2 rheometer (TA Instruments, New Castle, DE).

Strain Sweep Test

Strain was swept logarithmically from 0.1 to 100% at 10 points per decade. This range was chosen to ensure the entire linear viscoelastic region was captured in the data. For this testing the frequency was held constant at 0.1Hz in order to avoid wave propagation at small gap heights [9]. Three samples were taken for each gap height (50μm, 400µm, and 800µm). Tests for each gap height were performed with one set on smooth plates and one on plates with sandpaper. This resulted in a total of 18 samples for the strain sweep testing. The linear viscoelastic region was determined by finding the strain associated with a less than 5% change in the storage modulus [10], and a slope of the storage and loss moduli of less than +/- 0.2.

Stepped Flow Testing

To determine the viscosity of PST, stepped flow tests were performed by sweeping the strain rate from 0.01 to 100 s^{-1} logarithmically with 10 points per decade. This region was chosen based on the Newtonian region found using the Cox-Merz rule, which applies to many polymers [11].

As is shown in Figure 5, the Newtonian region is found at the beginning of the test and then tapers off around $1s^{-1}$. For this reason, the range of strain rates was centered around $1s^{-1}$.

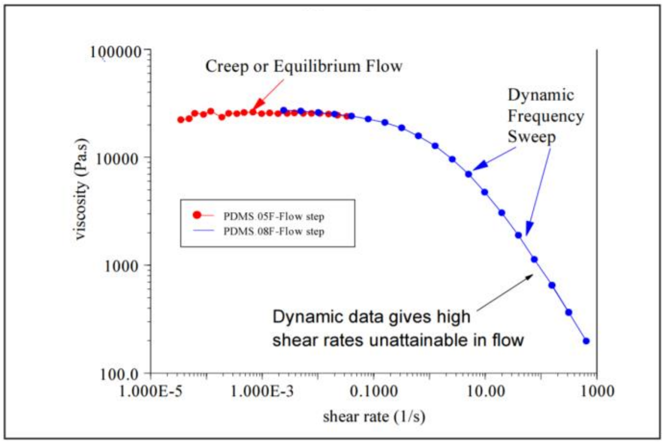


Figure 5. Stepped flow results for PDMS from literature [11]

Similar to the strain sweep tests, three samples were taken for each of the three gap heights (50µm, 400µm, and 800µm) both with smooth plates and on plates with sandpaper. This resulted in a total of 18 samples for the stepped flow tests. Each sample was analyzed to determine the viscosity by calculating the percentage change in the instantaneous viscosity between data points. The viscosities with less than a 1% change were extracted from the points before 1s^{-1}.

Frequency Sweep Testing

Frequency testing was performed in order to ensure that differing volumes produced no effect on the storage (G’) or loss moduli (G’’). They also served to determine if a gap correction factor was needed. Frequency tests were performed by sweeping from 0.01 to 100 Hz logarithmically with 10 points per decade. The strain was held constant at 2.51%, based on the results from the strain sweep tests. Three samples were taken at a 50µm gap and a 400µm gap for both a 10mm and a 20mm diameter plate set. This created a total of 12 samples for the frequency sweep tests. The 20mm diameter plate set was created in an identical manner to that for the final 10mm diameter plate set specified above. The frequency tests were performed using smooth plates only after it was identified in the stepped flow testing that there was no significant difference between the viscosities produced by plates covered with sandpaper and those without sandpaper at 400µm and 800µm gap heights, and even reduced data reliability at 50µm.

RESULTS

Strain Sweep

All tests exhibited a distinct linear region that encompassed a small range of strains. However, data from the 50µm gap height had a much larger linear region which resulted in a wider range of strains that represent the linear viscoelastic region. After looking at all the data, 2.51% was selected as a common value across all tests (Figure 6). This value was then used in the frequency sweep tests.

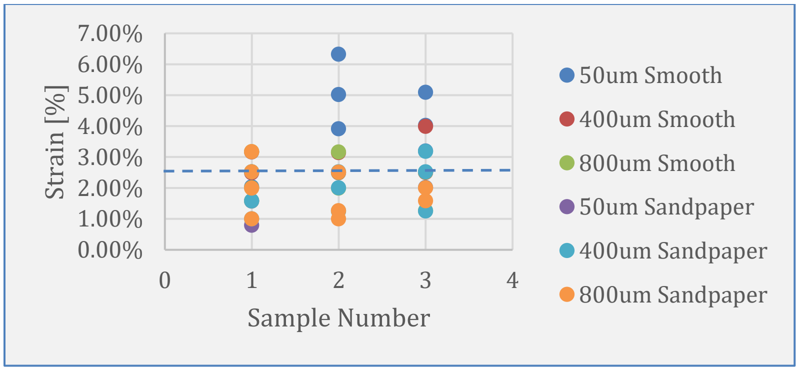


Figure 6. Compiled viscoelastic strain values from strain sweep testing

Stepped Flow

When more than one viscosity emerged from the extraction of data where the viscosity changed by less than 1%, the viscosity with the lowest percentage change was selected. Those sample viscosities were then averaged to determine an overall viscosity of 735 Pa*s. The results for the stepped flow tests for 50μm gap heights using sandpaper were removed from this average because, though their viscosities were in the expected range for two of the three samples, graphically their results did not match the pattern shown in the rest of the data set as shown in Figure 7.

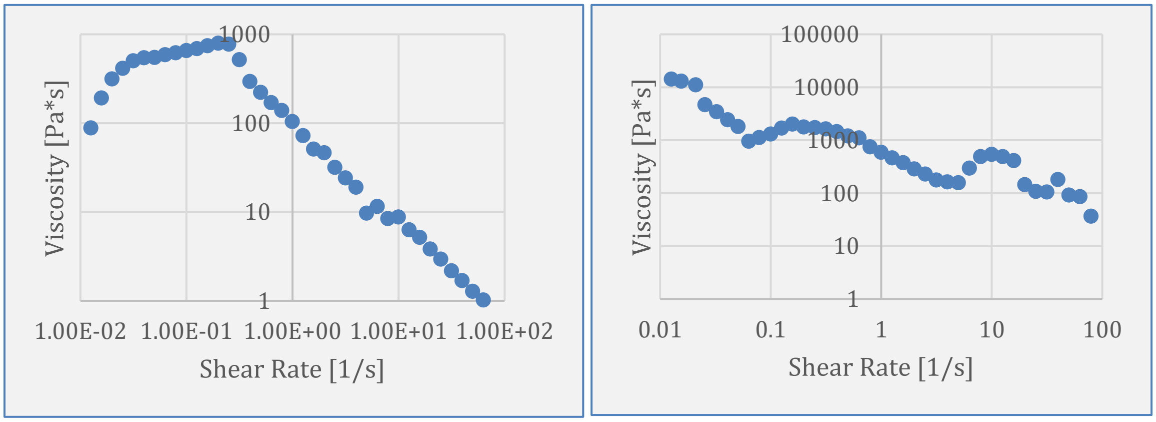


Figure 7. Stepped flow results with sandpaper at 400μm gap heights (left) and 50μm gap heights (right)

Typical stepped flow data from the study is shown in Figure 7 (left), which represents a test with a 400µm gap height and sandpaper grips. Results from stepped flow tests at 50µm gap heights with sandpaper did not exhibit typical flow behavior (Figure 7, right).

Frequency Sweep

The results of the frequency sweeps are presented in their entirety in Figures 8 and 9. Since sandpaper was not used in the frequency sweeps, it was not necessary to calculate a gap correction factor.

From these figures it can be seen that there is excellent overlap of G’ and G’’ at different gap heights and different volumes, with one exception. G’ and G’’ for the 10mm diameter plates at a 400µm gap height appeared lower than the other data sets.

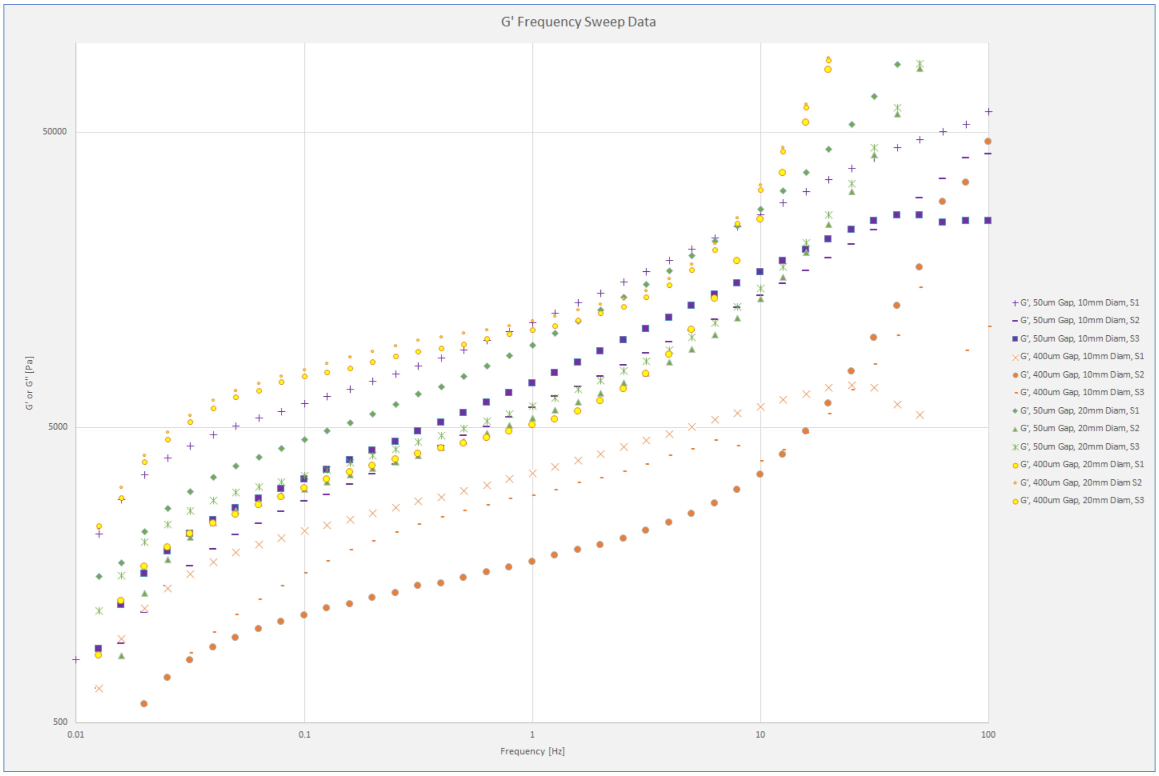


Figure 8. Storage modulus results from frequency testing

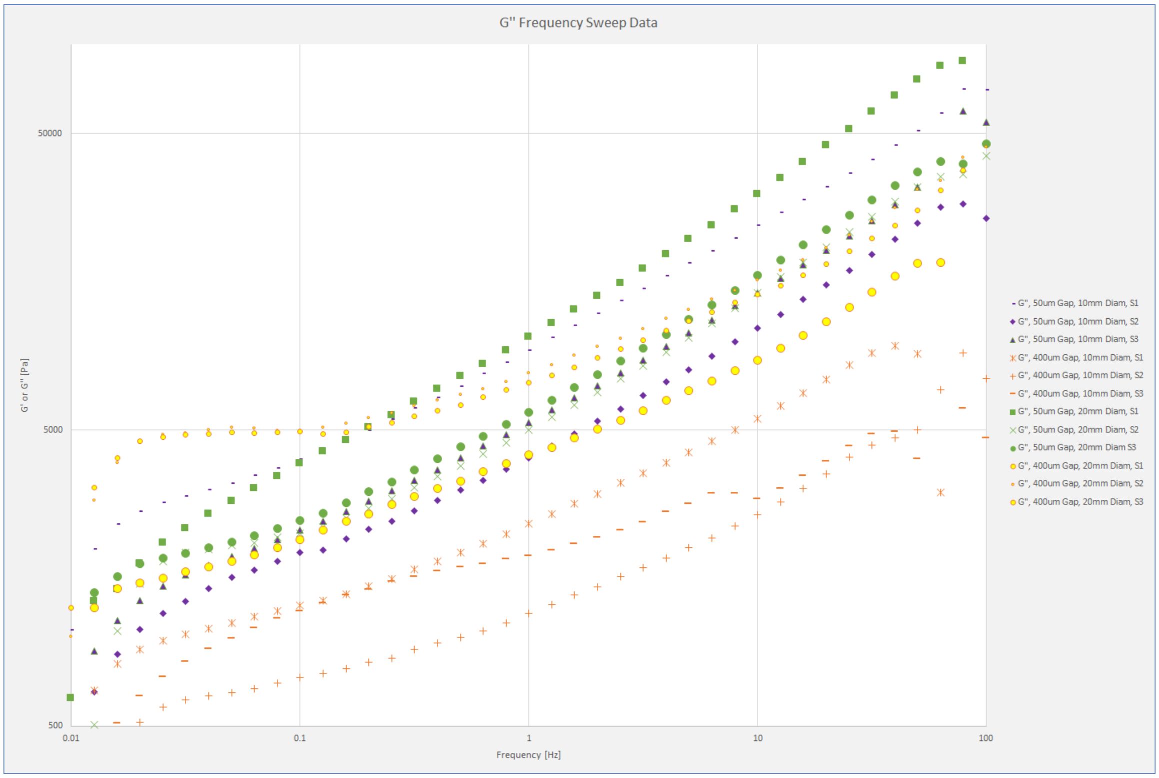


Figure 9. Loss modulus results from frequency testing

DISCUSSION

Initial data in this research had to be discarded after the many setbacks encountered in the early stages of experimentation. It was quickly learned that at gap heights on the magnitude of 50µm, the tolerance for the contact surface of the rheometer plates is critical to the accuracy of the data. It took a larger data set to be certain that data from these sets was out of pattern, but the stepped flow results were the deciding factor in determining that there was a measurable difference between gap heights when using common FDM 3D printing. Results significantly improved when the 3D printed top plate was replaced with an aluminum version.

Though a more niche obstacle, it is notable that a similar improvement in results was seen when ensuring that the bottom plate had a matching geometry to that of the top plate. This is because matching a matching diameter corrected the fill shape between plates. Here, acceptable levels of tolerance were attained by 3D printing the bottom plate with the contact surface facing the bed of the printer and replacing the part after 10 tests were performed with the use of toluene.

The use of sandpaper was determined to produce little effect in this study, with the exception of the 50μm gap height. At the smaller gap height, the sandpaper produced stepped flow data with magnitudes similar to other gap heights at times (some results were magnitudes higher than others) but graphically did not follow the expected flow behavior. This pattern held true regardless of the grit of the sandpaper used. This effect may be due to errors in zeroing the gap height when sandpaper has been applied. The grains may have hit each other rather than the paper backing when zeroing the rheometer, creating a zero height that is higher than it should be. Conversely, grains could be meshing down into the paper, creating a gap height measurement that is lower than it should be. Grains could also be changing shape as the plates are zeroed, the normal force from the zeroing process causing the grains to flatten or break off. In addition, the paper itself could be compressing as a result of the normal force, and on a scale of 50μm that paper compression could make up a significant proportion of the gap height. Regardless of the reasoning, sandpaper can enter errors into the data at 50μm gap heights and should be avoided until further examination of this effect can be performed. Roughing of a metal contact surface directly could mitigate some of these possible contributions to the effect and still provide increased grip.

The data from testing proved to be acceptably reliable after the errors from sandpaper and plate tolerancing were resolved. The data from the strain sweep tests seemed to form similar graphical relationships between gap heights and had similar linear viscoelastic regions between samples at different gap heights. The data from the stepped flow tests required the most correction, but eventually yielded viscosities in a relatively small range, with much crossover and similarity between sandpaper and smooth tests and different gap heights. The only exception being for the sandpaper data at 50µm, which data had to be discarded as it could not be corrected regardless of the sandpaper grit size. Data from the frequency tests showed very similar G’ and G’’ for differing gap heights and plate diameters, with the exception of the data for the 400µm gap height with a 10mm diameter plate set. It is difficult to speculate a cause for this exception, but could be explained by the plate diameter recommendation from TA instruments [12] for high viscosity fluids of 20 to 25mm. According to this recommendation, a 10mm plate could be undersized and result in the deviations found in the frequency sweep results. For volumes near the requirement of this experiment of 4.4µL, a 20mm diameter plate was not chosen because it would require a gap height less than 50µm and would risk greater noise in the results. At 400µm gap heights, using a larger 20mm diameter plate may have greater accuracy than a 10mm diameter plate.

LIMITATIONS AND FUTURE WORK

Though this experiment was performed with small volumes of vitreous in mind, it is important to note that vitreous testing has not yet been completed with this methodology. Commonly vitreous has a much lower viscosity than PST and will require alterations to this methodology. Such alterations might include increasing the plate diameter or adjusting the gap height. One alteration that should be implemented to improve the data overall and ensure data reliability is to machine the bottom plate out of aluminum as well. Another consideration is that whereas PST does not absorb into sandpaper, vitreous may do so and thereby alter the properties of the sandpaper and dehydrate the vitreous (in addition to the other complications involved with the use of sandpaper at low gap heights). If vitreous does not adhere to the plates as well as PST, then some form of cleats will be needed. One possible alternative to sandpaper may be to roughen the aluminum contact surfaces.

Referring back to the out of pattern data with 10mm diameter plates at 400µm gap heights from the frequency tests, it is important to note that the purpose of this research is to determine the effectiveness of parallel plate rheology on testing samples with a volume of 4.4µL. This deviation at larger gap heights does not seem to have any effect on the results of the frequency test at the 50µm gap height that would be appropriate for this volume.

CONCLUSION

Through the course of this experiment, it was determined that the use of sandpaper cleats did not have any major effects on the results of testing, except when used in a stepped flow test at 50µm gap heights, where it produced unexpected flow behavior and occasionally high viscosity. For this reason, it is recommended that in further experimentation sandpaper cleats should not be utilized for small volume rheology with a gap height of 50µm. It was also discovered that at 50µm gap heights, the tolerance of the plates becomes a significant concern and requires accuracy above that attainable by common FDM 3D printers. Aluminum plates are recommended to increase the machinability of the parts in order to improve the tolerances.

The results of the strain sweep tests showed a consistent linear viscoelastic strain of 2.51% across all gap height variations. The results of the stepped flow tests yielded a viscosity average in the area of 735 Pa*s when the data for 50µm gap heights with sandpaper is excluded. The results of the frequency sweep tests with smooth plates showed that there is no significant effect of plate diameter or gap height on G' and G'' with the exception of 10mm diameter plates at 400µm gap heights. This may be a result of slip produced by a small diameter plate at higher gap heights, but has little bearing on testing at 50µm gap heights as would be needed for parallel plate testing for mouse vitreous.

The viscosity of vitreous is commonly much lower than the viscosity of PST that was found here. TA Instruments recommends larger diameter plates for lower viscosity fluids [12]. For this reason, it will likely be necessary to use a larger diameter plate. However, a larger diameter plate will require a smaller gap height than 50µm for the tests to be performed properly. This may produce increasing noise, as found in preliminary testing. However, machining the bottom plate out of aluminum and tightening tolerances, as was done with the top plate, could mitigate these effects by reducing the noise produced at smaller gap heights and provide reliable results.

REFERENCES

[1] Lee, B., 1992, “Comparative rheological studies of the vitreous body of the eye,” Dissertation, Biomechanics, University of Pennsylvania.

[2] Colter, J., and Williams, A. and Moran, P. and Coats, B., 2014, “Age-related changes in dynamic moduli of ovine vitreous,” Journal of the mechanical behavior of biomedical materials, 41.

[3] Tram, N. K., and Swindle-Reilly, K. E., 2018, “Rheological Properties and Age-Related Changes of the Human Vitreous Humor,” Front Bioeng Biotechnol., 6(199).

[4] Silva, A. F. and Alves, M. A. and Oliveira, M. S. N., 2017, “Rheological behaviour of vitreous humour,” Rheologica Acta, 56, pp. 377-386.

[5] Kaplan, H.J. and Chiang, C.-W. and Chen, J. and Song, S.-K., “Vitreous Volume of the Mouse Measured by Quantitative High-Resolution MRI.” *Investigative Ophthalmology & Visual Science*, vol. 51, issue 13, April 2010.

[6] Nickerson, C.S., Kornfield, J. A., “The ‘Cleat’ Geometry: A Novel Rheological Tool.” *Journal of Rheology*, vol. 49, July 2005.

[7] Grainger Editorial Staff, 2017 (rev 2021), “Sandpaper Grit Charts & Grades.” From https://www.grainger.com/know-how/equipment-information/kh-sandpaper-grit-chart

[8] Garcia, M. T. and Gracia, I. and Duque, G. and de Lucas, A. and Rodriguez, J. F., 2009, “Study of the solubility and stability of polystyrene wastes in a dissolution recycling process,” Waste Management, 29 (6), pp 1814-1818.

[9] Ewoldt, R. H. and Johnston, M. T. and Caretta, L. M., “Experimental challenges of shear rheology: how to avoid bad data.” *Complex Fluids in Biological Systems*, 2014, pp 207-241.

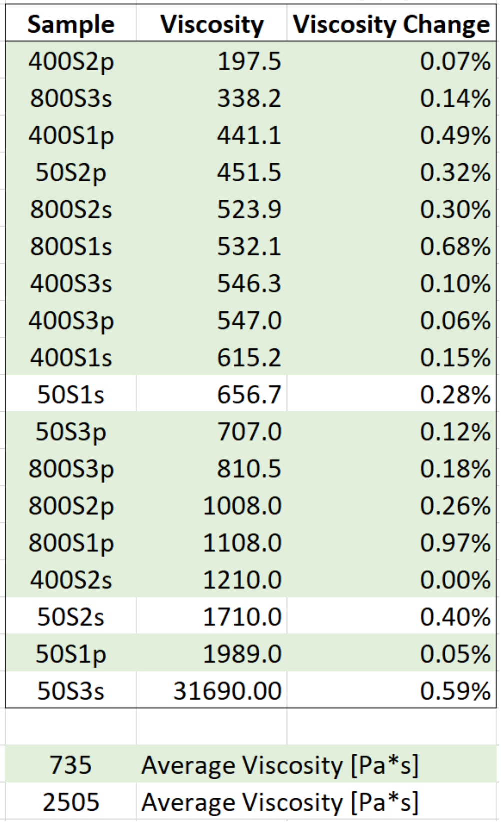
[10] TA Instruments, “Determination of the Linear Viscoelastic Region of a Polymer using a Strain Sweep on the DMA 2980.” From https://www.tainstruments.com/pdf/literature/TS61.pdf

[11] TA Instruments, “Rheology Applications Note – The Principles and Applications of the Cox-Merz Rule.” From http://www.tainstruments.com/pdf/literature/RN14.pdf

[12] TA Instruments, “Rheology Theory and Applications.” From https://people.clarkson.edu/~skrishna/DHR_Rheology_Theory.pdf

APPENDIX

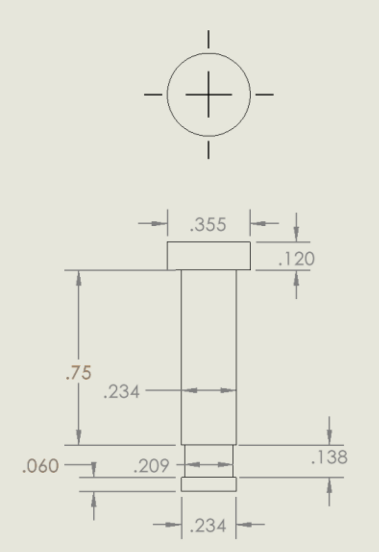
A1 – Stepped flow data



Sample	Viscosity	Viscosity Change
400S2p	197.5	0.07%
800S3s	338.2	0.14%
400S1p	441.1	0.49%
50S2p	451.5	0.32%
800S2s	523.9	0.30%
800S1s	532.1	0.68%
400S3s	546.3	0.10%
400S3p	547.0	0.06%
400S1s	615.2	0.15%
50S1s	656.7	0.28%
50S3p	707.0	0.12%
800S3p	810.5	0.18%
800S2p	1008.0	0.26%
800S1p	1108.0	0.97%
400S2s	1210.0	0.00%
50S2s	1710.0	0.40%
50S1p	1989.0	0.05%
50S3s	31690.00	0.59%
735	Average Viscosity [Pa*s]	
2505	Average Viscosity [Pa*s]	

Viscosity values for each stepped flow test performed, labeled in the form [gap height in µm, sample number, parallel (p) or parallel with sandpaper (s)]. The viscosities are organized from lowest to highest. Values used in the average are highlighted in green with the actual viscosity (735 Pa*s) highlighted in green below the results. The data for 50µm gap heights with sandpaper were removed and are not highlighted in green, but the viscosity had they been included is displayed below the chart without a green highlight (2505 Pa*s).

A2 – Machined 10mm plate drawings (IPS)



The drawing for the 10mm diameter plate as specified in IPS units for ease of manufacturing in most machine shops in the United States of America. The 20mm plate is identical with the exception of the diameter of the contact surface (0.355in in in the drawing here) which was machined at 0.787in in diameter.

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