MICROFLUIDICS MEETS DILUTE SOLUTION VISCOMETRY:

An Undergraduate Laboratory to Determine Polymer Molecular Weight Using a Microviscometer

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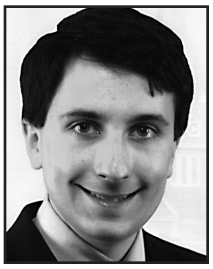
Fluid viscosity is an important fluid property to monitor in industry, research, and medicine. The diverse applications for the rapid measurement of fluid viscosity include the characterization of inks in ink-jet printing, [1] studies of protein dynamics,[2] the characterization of biomaterials used in drug delivery such as hyaluronic acid (HA), [3] and the clinical detection of diseases such as paraproteinemia[4] and ischemic heart disease[5] through the study of blood. An additional use of viscometry is in the determination of the hydrodynamic volume and molecular weight of macromolecules. Using the data analysis seen later in this paper, a polymer's molecular weight can be estimated. It is important to be able to measure a polymer's molecular weight—because of its impact on such properties as strength, stiffness, and glass transition temperature—by simply measuring the viscosity of dilute polymer solutions of varying concentrations.

In a laboratory setting, viscosity measurements of dilute polymer solutions are typically made with glass capillary viscometers such as Ubbelohde viscometers that require mL of fluid for measurement. The development of microfluidic viscometers[6-9] means that such viscosity measurements can now be quickly made with only μL of fluid. Microviscometers can thus potentially be used to determine the molecular weight of polymer samples even when sample volumes are severely limited.

To illustrate both the use of microfluidics to determine fluid viscosity and the use of dilute solution viscometry to determine polymer molecular weight, we developed a low-cost laboratory procedure for students to use PDMS microviscometers to determine the molecular weight of a polymer sample. In addition to the procedure, we present sample data for microviscometer tests run on glycerol solutions and on samples of PEO that match up well with viscometry results obtained with conventional Ubbelohde viscometers. We also discuss the timing and logistics of the lab and the feedback obtained from two sample laboratory sessions run with undergraduates.



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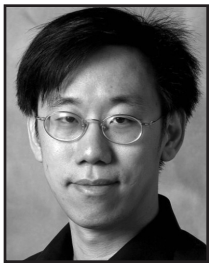

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MATERIALS

For soft lithography microchannel fabrication, SU-8 2050 negative photoresist and SU-8 developer were acquired from Microchem (Newton, MA). Sylard-184 poly(dimethylsiloxane) (PDMS) was obtained from Dow Corning (Midland, MI) and 1,1,2-trichlorosilane (T2492) (a release agent) was obtained from United Chemical Technologies (Bristol, PA). Samples of PEO with viscosity average molecular weights of ~1 MDa and ~4 MDa were obtained from Sigma-Aldrich (St. Louis, MO). Aqueous solutions of the 1 MDa PEO were prepared by mixing the solutions with a stir bar overnight. Experiments to determine the viscosity of these solutions were performed within eight days of when the solutions were prepared. An aqueous solution of 3 mg/mL of the 4 MDa was prepared by stirring the solution for three days. The shear thinning studies performed using this solution were performed within one day of when the solution was prepared. Glycerol from Fisher Scientific (Pittsburgh, PA) was used to prepare aqueous glycerol solutions.

METHODS

Device Fabrication

Microfluidic viscometers (PDMS channel on PDMS flat substrate) were fabricated using the rapid prototyping technique.[10] Briefly, the viscometer device was designed using AutoCAD (Autodesk, San Rafael, CA). A silicon-SU-8 master was created using conventional UV photolithography (with the SU-8 layer being 55 μm). After surface treatment of gas-phase 1,1,2-trichlorosilane (a release agent) on the master, a degassed 10:1 mixture of PDMS precursor and curing agent was then cast onto the master (about 2.5 mm thick—thickness not critical). After being cured at 70 °C for at least two hours, the PDMS slab was peeled from the master and cut into devices. A flat PDMS slab and the PDMS piece with the channel imprints were then treated for 30 seconds in an air plasma (Harrick Plasma, Ithaca, NY)

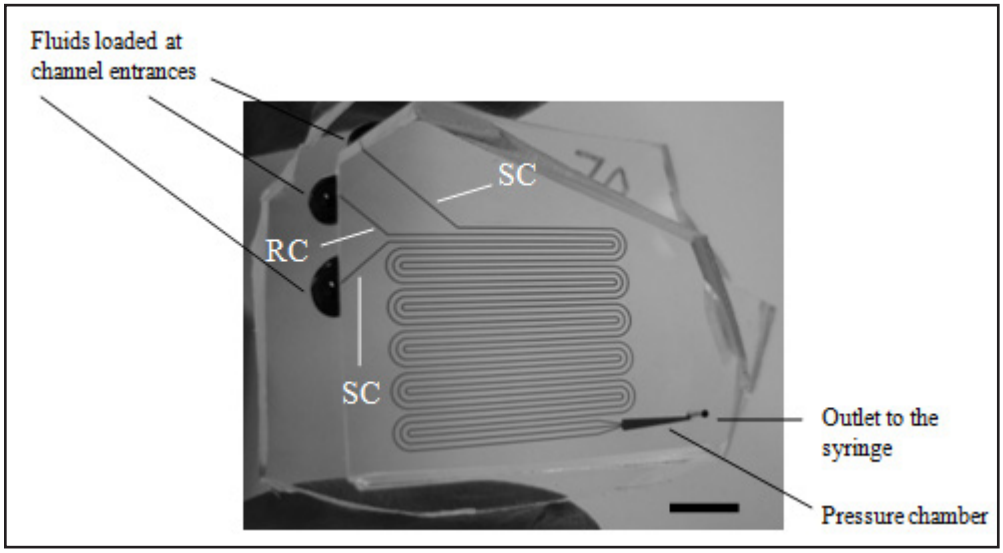

Figure 1. *The PDMS viscometer with two sample channels (SCs) and one reference channel (RC) for fluid flow. The device was filled with dye for visual effect. Scale bar is 5 mm.*

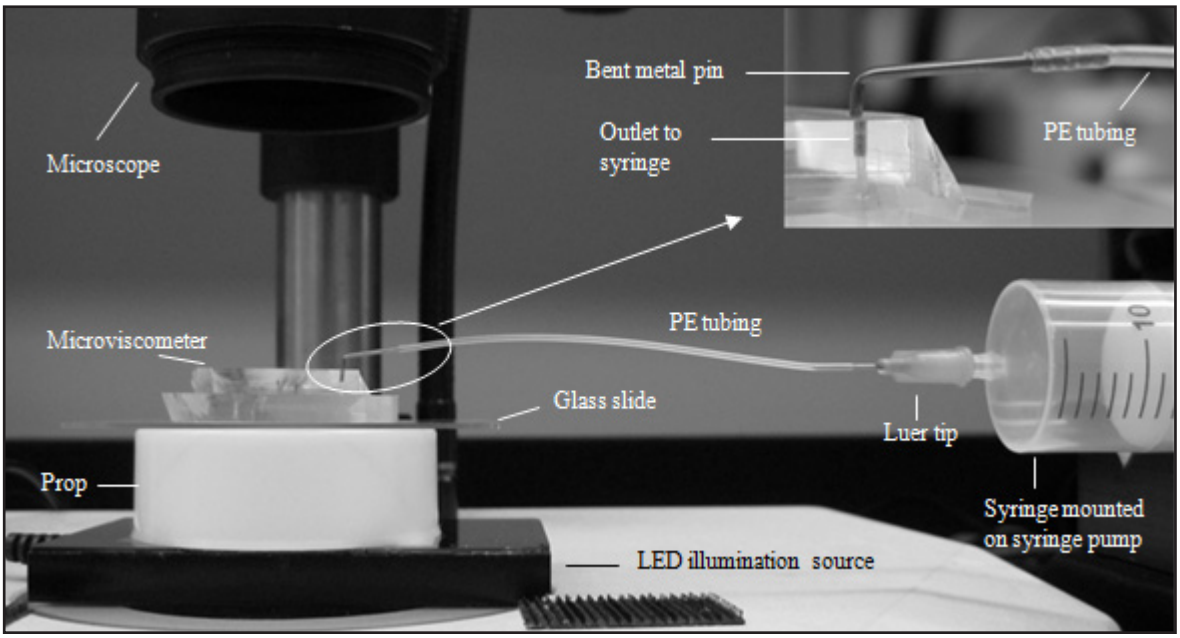

Figure 2. *Setup for using the microviscometer. After the syringe pump is turned on to pull the syringe back, a camera attached to the microscope is used to record the movement of fluids through the viscometer.*

and bonded together to form the PDMS viscometer (Figure 1). Tests were not run on the viscometers until at least two days after their fabrication to reduce the hydrophilicity of the device channels.

Experimental Setup

The PDMS viscometer consisted of three channels of height h ~ 55 μm, width w ~ 100 μm, and length L_{total} ~ 20.4 cm. The viscometer was prepared for use by using micropipettes to place two drops of sample fluids and one drop of a reference fluid of known viscosity at the entrances of the three channels in the top left of the device. A syringe pump (Harvard Apparatus, Holliston, MA) was then used to generate a sub-atmospheric pressure within the device channels to drive flow. A syringe attached via a Luer stub and polyethylene tubing (Scientific Commodities, Inc., Lake Havasu City, AZ) to a bent hollow metal pin was first placed in the pump and the metal pin was inserted into the pressure inlet in the bottom right of the device (Figure 2). The syringe pump was then used to pull the syringe at a constant rate while the flow through the channels was tracked with a Moticam 2300 camera (Motic, Xiamen, China) mounted on a Stemi SV11 dissecting microscope (Zeiss, Obercochen, Germany). The transparent liquids moving through the viscometer caused contrast with the background to decrease as the liquids passed through them (Figure 3).

The videos taken from the tests were analyzed with MATLAB to track the length of each fluid stream over the duration of the test. For the tests on PEO described below, the videos had a frame rate of 13 to 16 fps and were analyzed every four frames. The code operates by subtracting previous images from each frame and detecting the movement of a stream as a change in grayscale intensity that surpasses a certain threshold. Adjacently marked pixels are combined to make up the three streams, and the length of each stream is then found by dividing the total number of pixels in that stream by a constant thickness value.

Mechanism and Theory of Microviscometer

This analysis of fluid flow follows that of Han, et al.,[6] since our method and theirs use Poiseuille flows through rectangular channels, differing mainly in the way the driving pressures are applied. The constant pulling of the syringe attached to the viscometer generates a continually decreasing pressure inside the channels of the device that is lower than the air pressure at the channel entrances. The laminar flow generated by this pressure can be described by the Hagen-Poiseuille equation[11]:

$$v = \frac{d_h^2}{S\eta}\frac{\Delta P}{L} \quad (1)$$

where v is the velocity of the fluid; d_h is the hydraulic diameter of the channel related to the height h and width w, d_h = 2hw / (h+w); η is the dynamic viscosity of the fluid; S is a constant related to channel geometry, with S = 32 for rectangular channels; ΔP is the pressure drop across the fluid; and L is the length of the advancing fluid front.

The pressure drop ΔP consists of two components, *i.e.*, $\Delta P = \Delta P_d + P_c$, where P_c is the capillary pressure. ΔP_d is the pressure difference between the fluid inlet, which is constantly at atmospheric pressure P_0, and the moving fluid front, which is at the constantly decreasing pressure inside the viscometer P_i, *i.e.*, $\Delta P_d(t) = P_0 - P_i(t)$. For a test where a sample fluid and a reference fluid are pulled through the viscometer at the same time, $\Delta P_d(t)$ is the same for the two streams and the following equations can be written using Eq. (1):

$$\frac{S}{d_h^2}\eta_s L_s(t) v_s(t) = P_0 - P_i(t) + P_{c,s} \quad (2)$$

$$\frac{S}{d_h^2}\eta_r L_r(t) v_r(t) = P_0 - P_i(t) + P_{c,r} \quad (3)$$

where the subscripts s and r refer to the sample and reference streams, respectively. Combining and integrating Eqs. (2) and (3) leads to the equation

$$\frac{L_r^2(t_2) - L_r^2(t_1)}{t_2 - t_1} = \frac{\eta_s}{\eta_r}\frac{L_s^2(t_2) - L_s^2(t_1)}{t_2 - t_1} + 2d_h^2\frac{P_{c,r} - P_{c,s}}{S\mu_r} \quad (4)$$

The value of $\frac{\eta_s}{\eta_r}$ for a given test was thus found by taking the slope of a linear fit of $\frac{L_r^2(t_2) - L_r^2(t_1)}{t_2 - t_1}$ vs. $\frac{L_s^2(t_2) - L_s^2(t_1)}{t_2 - t_1}$ where $L_r(t)$ and $L_s(t)$ were determined from the processing of each video. For the tests on PEO described below, an interval of five frames was used for the time interval $t_2 - t_1$.

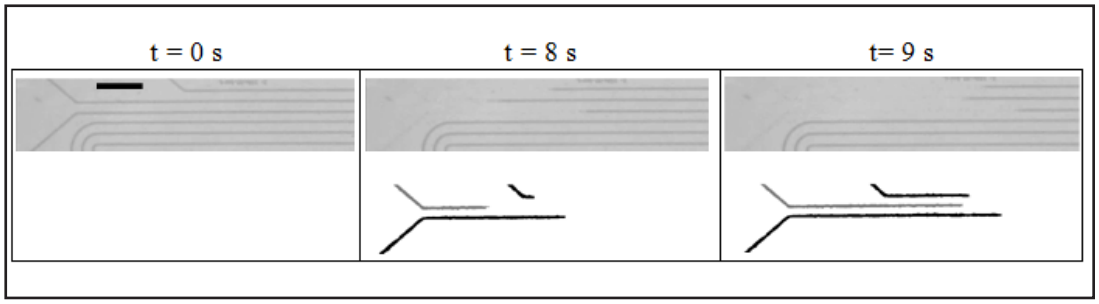

Figure 3. *Microphotographs of the beginning of a viscometry test run with water and PEO solutions (top row) and the output of the MATLAB code used to track the movement of each stream (bottom row). Scale bar is 2 mm.*

Dilute Solution Viscometry

For dilute polymer solutions, the addition of higher concentrations of polymer leads to higher solution viscosities in accordance with the Huggins equation[12]

$$\frac{\eta_{sp}}{c} = [\eta] + k[\eta]^2 c \qquad (5)$$

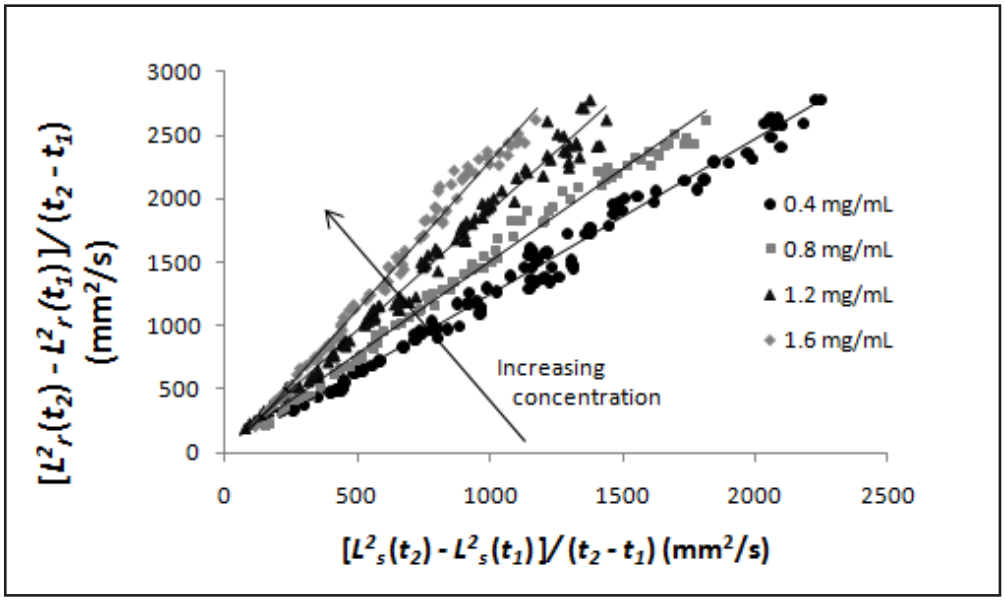

Figure 4. *Sample plots of $[L^2_r(t_2) - L^2_r(t_1)]/(t_2 - t_1)$ vs. $[L^2_s(t_2) - L^2_s(t_1)]/(t_2 - t_1)$ for aqueous 1 MDa PEO solutions of different concentrations. The relative viscosity of each solution is found as the slope of its linear fit.*

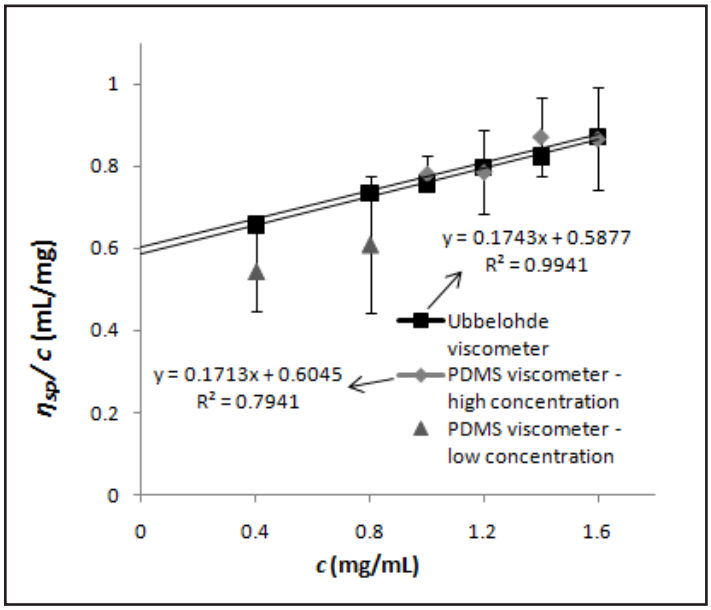

Figure 5. *Plots of η_{sp}/c vs. c used to determine values of $[\eta]$ for the 1 MDa PEO sample using viscosity data from the Ubbelohde viscometer and the PDMS viscometers. Linear fits are shown from which $[\eta]$ values were determined as the intercepts. Only the four highest concentrations were used in the linear fit for the PDMS viscometers. Error bars represent the standard deviation of η_{sp}/c.*

where η_{sp} is the specific viscosity of a polymer solution of concentration c, defined as $\eta_{sp} = \frac{\eta_{solution}}{\eta_{solvent}} - 1$ where $\eta_{solution}$ is the viscosity of the polymer solution and $\eta_{solvent}$ is the viscosity of the pure solvent; $[\eta]$ is the intrinsic viscosity of the polymer solution and is a representation of the hydrodynamic volume that the polymer chains take up in solution, and k is Huggins' constant. If the viscosities of different concentrations of a polymer in solution are known, then a value of $[\eta]$ for the polymer-solvent pair can be found as the intercept of a graph of $\frac{\eta_{sp}}{c}$ vs. c. The value of $[\eta]$ can then be related to molecular weight using Mark-Houwink relation[12]: $[\eta] = KM^a$, where M is polymer molecular weight and K and a are empirical Houwink constants for a given polymer-solvent pair. The values of K and a are known for many common polymers including PEO, having been determined experimentally by measuring values of $[\eta]$ for a polymer at known molecular weights. For polymers with a molecular weight distribution, the measured value of M through this method is an average known as the viscosity average molecular weight M_v, typically between the number-average M_n and the weight-average M_w.

Ubbelohde Viscometry

Macroscale viscosity measurements of the glycerol and PEO solutions for validation purpose were made with a Cannon Ubbelohde viscometer of diameter 0.58 mm (State College, PA) in a water bath of 23.0 ˚C. Twelve mL of fluid were needed for each test. Water was used as the reference fluid in the tests. The relative viscosity of each glycerol solution was found by multiplying the ratio of efflux times of the solution and the pure solvent by the (measured) density of that solution. Density differences between the dilute PEO solutions and water were negligible, so the relative viscosity of each PEO solution was found simply as the ratio of the efflux times of the solution and the pure solvent.

VALIDATION OF THE DEVICE OPERATION

To ensure that the microviscometer produced accurate viscosity readings, tests were first run on the device using glycerol solutions as sample streams and water as the reference stream. Pressure was generated with a 50 mL syringe that was pulled at rates ranging from 3.50 mL/min to 21.84 mL/min. Tests were performed at room temperature averaging ~ 23 °C. The viscosities of the glycerol solutions were measured with an Ubbelohde viscometer in a 23.0 °C bath for comparison (Table 1). The results from the microviscometer are seen to be consistent with the Ubbelohde viscometer although the variance in the microviscometer tests is much higher.

Viscosity measurements were then made with the microviscometer using dilute 1 MDa PEO solutions as sample streams and water as the reference stream. For these tests, pressure was generated by pulling a 50 mL syringe at an initial volume of 25 mL at a rate of 5.46 mL/min. Note that the exact initial volume of the syringe and the pulling rate used in the experiments are not critical, as the viscometer can function over a range of generated pressure gradients. Pressure-induced deformation of the microchannels could occur in a PDMS device such as ours if pressure differences were too large but the maximum pressure gradients across the channels in these experiments were only ~15 kPa for the glycerol tests and ~10 kPa for the PEO tests. No deformation of the channels was observed under the microscope in any test.

The PEO tests were performed at 23.0 °C ± 0.5 °C and the measured viscosity values were compared to values obtained with an Ubbelohde viscometer in a 23.0 °C bath (Table 1). Sample plots of

$$\frac{L_r^2(t_2)-L_r^2(t_1)}{t_2-t_1} \text{ vs. } \frac{L_s^2(t_2)-L_s^2(t_1)}{t_2-t_1}$$

used to calculate viscosity values in the microviscometer tests are seen in Figure 4.

In a few of the microviscometer tests, PEO solutions began to flow through the viscometer before the syringe was pulled, suggesting that the PEO solutions had a positive value of $P_{c,sample}$, *i.e.*, they wet the PDMS surface. This did not interfere with data collection, however, and the results from the viscometer were still valid for times while all fluids were moving.

It can be seen from Table 1 that the viscosities of the 1 mg/mL, 1.2 mg/mL, 1.4 mg/mL, and 1.6 mg/mL solutions measured by the microviscometer matched the results from the Ubbelohde viscometer well while the viscosities of the 0.4 mg/mL and 0.8 mg/mL solutions measured by the microviscometer were somewhat lower than that of the Ubbelohde viscometer, possibly due to the high surface areas of microdevices and loss of polymer from the solution to the surface. The variance for the microviscometer is seen to be much greater than that for the Ubbelohde viscometer at all concentrations, which may be due to image processing errors or to the much smaller sample size.

The viscosity results from the PDMS viscometers and the Ubbelohde viscometer were then used to find values of $[\eta]$ for the PEO sample by plotting $\frac{\eta_{sp}}{c}$ vs. c and taking $[\eta]$ as the y-intercept (Figure 5). The Ubbelohde viscometer data extrapolated to a value of $[\eta] = 0.588$ mL/mg. When all the data for the microviscometer were used, a much lower value of $[\eta] = 0.424$ mL/mg was found (extrapolation not shown). This discrepancy in $[\eta]$ values is caused by the lower viscosities found with the microviscometer at lower c: the error in the plot of $\frac{\eta_{sp}}{c}$ is magnified for smaller c, which also corresponds to larger differences in η_{sp}.

To reduce the error in $[\eta]$ estimation, low concentrations of polymer solution should be avoided in the experiments. As shown in Figure 5, excluding the 0.4 and 0.8 mg/mL microviscometer data from the extrapolation results in an extrapolated value of $[\eta] = 0.605$ mL/mg, which agrees well with the values from Ubbelohde experiments.

TABLE 1

Relative viscosity values determined for aqueous solutions of glycerol and PEO vs. water using an Ubbelohde viscometer and PDMS viscometers. Each solution was measured three times with the Ubbelohde viscometer and multiple times with the PDMS viscometers as marked.

—	$\frac{\eta_{solution}}{\eta_{solvent}}$ ± standard deviation		—
Solution	Ubbelohde viscometer	PDMS viscometer	Number of microviscometry trials
10 % glycerol	1.25 ± 0.003	1.32 ± 0.05	10
20 % glycerol	1.77 ± 0.003	1.80 ± 0.13	12
30 % glycerol	2.38 ± 0.015	2.37 ± 0.12	18
50 % glycerol	6.01 ± 0.012	6.07 ± 0.64	12
0.400 mg/mL PEO	1.26 ± 0.0009	1.22 ± 0.04	5
0.800 mg/mL PEO	1.59 ± 0.002	1.49 ± 0.13	5
1.00 mg/mL PEO	1.76 ± 0.003	1.78 ± 0.05	5
1.20 mg/mL PEO	1.96 ± 0.006	1.94 ± 0.12	5
1.40 mg/mL PEO	2.15 ± 0.005	2.22 ± 0.13	5
1.60 mg/mL PEO	2.40 ± 0.012	2.39 ± 0.20	5

Using values of a = 0.78 and K = 12.5 * 10^{-6} mL/mg $(g/mol)^{1/a}$ for aqueous PEO solutions[13] and the $[\eta]$ values above, the Mark-Houwink equation produces values of M = 1,010,000 g/mol for the PDMS viscometers and M = 977,000 g/mol for the Ubbelohde viscometers. These values are in good agreement with each other as well as with the value reported by the manufacturer.

LABORATORY IMPLEMENTATION, COST AND LOGISTICS, AND STUDENT FEEDBACK

Laboratory Implementation

The laboratory procedure consists of a device fabrication demonstration, student-run microviscometer tests on PEO solutions, image processing of the tests using MATLAB, and a shear-thinning demonstration. After the lab session, viscosity data from different students can be combined and analyzed to find an estimate for the molecular weight of the PEO sample used. If time is available, students can also measure the viscosities of the PEO solutions with macro viscometers such as Ubbelohde viscometers to validate the microviscometer data. This allows students to visualize the advantages and disadvantages of microviscometry in terms of accuracy, precision, speed, cost, and fluid volume required.

Two trials of this procedure were run with volunteer undergraduates (mostly junior students who have taken transport phenomena) from the Georgia Institute of Technology School of Chemical & Biomolecular Engineering. Each trial had four students with no microfluidics experience who performed the viscometer tests and the first trial had an additional three students who had worked in a microfluidics laboratory before. Several days before the laboratory sessions were held, students were provided with a copy of the procedure as well as a "pre-lab" that provided the background, theory, and a quiz to test their understanding prior to the lab. The beginning of the laboratory consisted of a microviscometer fabrication demonstration given by the undergraduate teaching assistant. The assistant explained how masks and masters are manufactured, explained how PDMS is mixed, cast, cured, and bonded to form devices, and used the plasma cleaner to bond a device to show to the students. If time allows, this simple micromolding step and device fabrication can be incorporated into the lab, and concepts such as cross-linking, Poisson ratio, Young's modulus, and surface treatment can be explained and demonstrated.

The students then ran two microviscometer tests where each test used two different concentrations of 1 MDa PEO as sample streams and water as the reference stream. Concentrations of 0.500, 1.00, 1.50, and 2.00 mg/mL were used in the two tests. Pressure was generated by pulling a 50 mL syringe at an initial volume of 25 mL at a rate of 5.46 mL/min (the same conditions as in the validation tests for the PEO solutions).

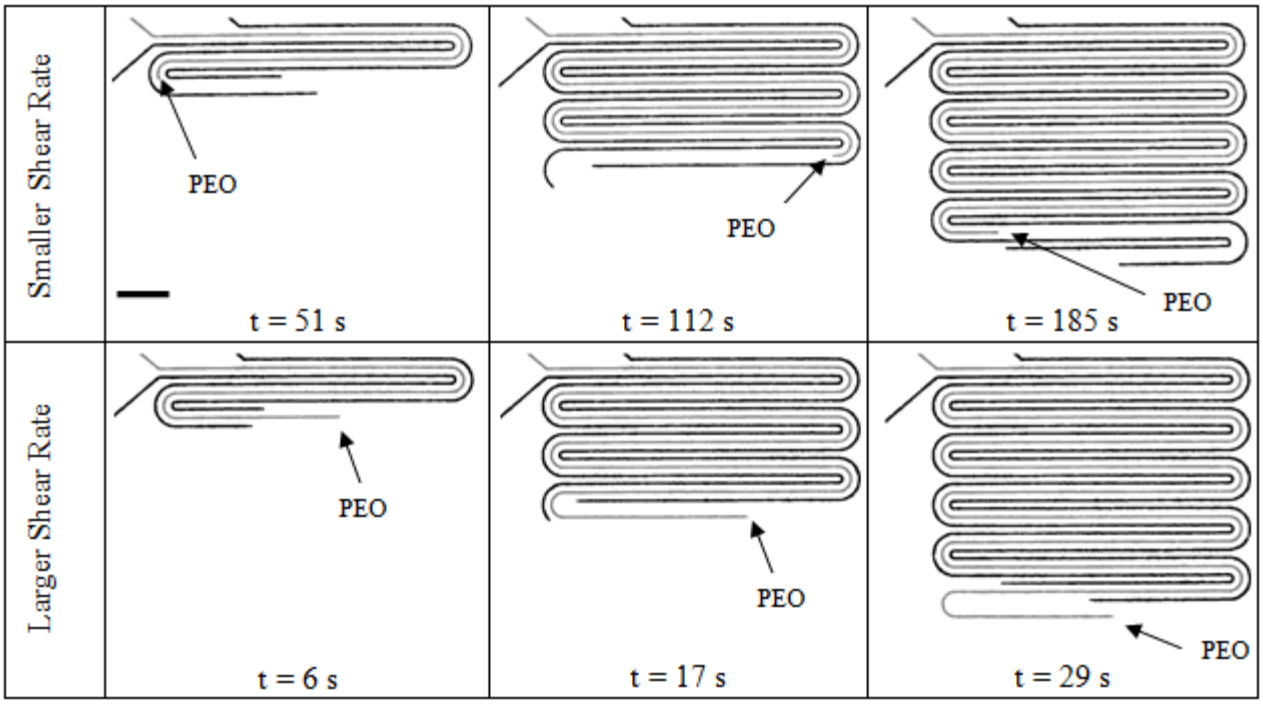

Figure 6. *Shear thinning display of 4 MDa PEO (middle channel, gray) vs. 60% glycerol (outer channels, black). The top row shows MATLAB output images of a viscometer test run at an average shear rate of ~ 100 s^{-1} at which the glycerol solution outraces the PEO solution. The bottom row shows images of a test run at a shear rate of ~ 780 s^{-1} at which the PEO solution has a lower viscosity than at the slower rate and outraces the glycerol solution. Scale bar is 3 mm.*

Image Processing

The students then used the pre-written MATLAB code to analyze their videos. In our experience, some of the trouble-shooting issues with the image processing can be explained to the students during the lab module to facilitate data processing. For instance, it is important to take a video that has both high contrast (for the streams to be located by the code) and uniform contrast (for the streams to be tracked with uniform width). Problems with noisy images can be addressed with MATLAB filtering of the raw video and with data smoothing of the acquired length values.

Demonstration of Shear Thinning Fluids

To demonstrate both the shear thinning behavior of non-dilute polymer solutions and the ability to generate a large range of shear rates in the viscometer using the syringe pump, the students then ran a test with a high pulling rate and a test with a low pulling rate on a sample of 3 mg/mL 4 MDa PEO with 60% glycerol solutions as reference fluids. When a test is run with a syringe initial volume of 40 mL and a pulling rate of 1.7 mL/min, corresponding to an average shear rate ~100 s^{-1}, the 60% glycerol reference is seen to move through the viscometer more quickly than the PEO solution (Figure 6). In contrast, the PEO solution is seen to move through the viscometer more quickly than the 60% glycerol reference when given a higher average shear rate of ~780 s^{-1} (generated by pulling a syringe at an initial volume of 5 mL at a rate of 20 mL/min). This inversion of behavior is caused by the lower viscosity of the PEO solution at a higher shear rate as opposed to the rate-independent viscosity of the Newtonian glycerol solution. The shear thinning behavior of the PEO solution over this range of shear rates was verified using a Physica MCR 3000 rheometer (Anton-Paar, Graz, Austria); the viscosity of the PEO solution fell from ~ 14 cP at 100 s^{-1} to ~ 8.6 cP at 780 s^{-1}. This method can be used to demonstrate non-Newtonian behaviors of various fluids in the range of shear rates up to 2000 s^{-1}.

Cost Estimate and Timing Logistics

Assuming that laboratory equipment such as microscopes, cameras, a plasma cleaner, and a syringe pump are available, the laboratory costs come in the materials. The fabrication of a mask and master costs around $150, and samples of the 1 MDa PEO, 4 MDa PEO, glycerol, and PDMS cost ~$30 each for a total startup cost of <$300. Note that other water-soluble polymers can be substituted for PEO if desired, and fluids other than glycerol solutions can be used as viscosity standards as long as they do not swell PDMS and their viscosity is known. If needed, we estimate that a simple microscope and camera setup are in the range of $2,000 to $3,000. If a plasma cleaner is not available, it is possible to create devices by pressing a flat PDMS slab against a PDMS slab with channel imprints, placing the slabs between two glass slides, and then holding the glass slides together using rubber bands.

Once the startup materials are present, the individual lab sessions have a very low cost because of the small volumes of chemicals needed. The major repeated cost is in fabricating the PDMS devices which consume ~$1.50 of PDMS per chip. Approximately 5 hours of time were devoted by the undergraduate teaching assistant to prepare for each lab session, including device fabrication, solution preparation, and lab set-up. The two lab sessions took about 1 hour and 45 minutes each to complete, including the fabrication demonstration, the completion of four viscometer tests, and the processing of the tests and the description of the MATLAB code.

Student Feedback

Students who participated in the laboratory experiments provided informal feedback. Most students found the module was effective in introducing the concept of solution viscometry and microfluidics, to which most of them had had no prior exposure. The students found more background on microfluidics and microfabrication details would be both more interesting and more useful. This suggests that the laboratory module should be expanded to multiple sessions to deal with the individual topics in depth. The students also commented that seeing non-Newtonian behavior with a real demonstration could reinforce this concept that they learned in the classroom.

CONCLUSIONS

We present a procedure for a student laboratory session to demonstrate the use of microfluidics to determine fluid viscosity and the use of dilute solution viscometry to estimate polymer molecular weight. Overall, the results were reasonably consistent with those found from conventional Ubbelohde viscometry. The laboratory also allows students to see firsthand how microfluidic devices are fabricated and to observe a visual demonstration of the shear thinning behavior of non-dilute polymer solutions. Assuming soft lithography equipment is available, the experimental setup is very quick and affordable. The laboratory serves as an excellent way to generate interest in the fields of polymers, rheology, and image processing while invigorating students with the opportunity to work hands-on in the "cutting-edge" realm of microfluidics.[14] The combination of written instruction in the pre-lab and procedure, verbal instruction and visual displays from the teaching assistant, and hands-on experience for each student caters to a range of different student learning styles.[15-16] Because it is multi-faceted, this experimental platform can be used and re-used in different pedagogical contexts, or it can be a problem-solving based learning tool.[17] We recommend running the following laboratory modules individually or in combination depending on the need of the curricula and time available for the laboratory experiments: (1) laminar flow – Hagen-Poiseuille relationship; (2) viscometry; (3) demonstration of non-Newtonian flow; (4) microfabrication; (5) other concepts of polymer processing; (6) image processing.

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