Undergraduate Laboratory Experiment

TEACHING FUNDAMENTAL CONCEPTS OF RHEOLOGY

In Context of Sickle Cell Anemia

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Biomedical engineering is a relatively new discipline that has emerged from the collaboration of engineers, physicians, and life scientists.[1] The biomedical industry has demonstrated explosive growth over recent years, and employment is expected to grow 72% between 2008 and 2018.[2] Thus, educational institutions need to provide undergraduate and graduate students with skills and knowledge relevant to this industry.

Since mass, heat, and momentum transfer are an essential part of living systems, understanding transport processes and their application is a critical component of the education of the biomedical engineer. For instance, knowledge of the rheological properties of blood is crucial for understanding the dynamics of the circulatory system[3] and blood-biomaterial interactions.[4] In the following hands-on experiment, fundamental principles in rheology are presented to the students within the context of sickle cell anemia, a condition that alters the flow characteristics of blood due to decreased flexibility of red blood cells (RBCs). Sickle cell disease is the most common inherited red blood cell (RBC) disorder, with more than 200,000 children affected every year.[5] Introducing students to concepts of rheology within the context of this significant disease is an example of an inquiry-based approach to teaching. This strategy been shown to improve student motivation to learn within a project-based setting.[6]

In this activity, students prepare two-phase blood analogs composed of chitosan particles suspended in aqueous glycerol solutions, which substitute for RBCs and plasma, respectively. The students also generate an analog composed of rigid chitosan particles, produced by crosslinking the chitosan with glutaraldehyde, simulating sickle cell blood. Lastly, the hematrocrit content, or cell volume, of the blood analogs is varied by controlling the amount of suspended chitosan particles. The flow properties of these analogs are examined

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with a rotational viscometer and data fit to the Casson Model, a constitutive relationship that describes the rheological behavior of two-phase systems. The objectives of this laboratory module are:

1. *To conduct a rheological characterization of Newtonian and non-Newtonian fluids and analyze and interpret the data.*
2. *To learn to quantify fluid viscosity and Casson parameters as a function of hematocrit content and RBC deformability.*
3. *To relate the physiological significance of these flow parameters to sickle cell anemia.*

Through this experiment students explore many concepts and tools that they will use throughout their engineering careers:

- *Novel application of transport phenomena and materials science—foundational chemical engineering principles*
- *Viscosity measurement*
- *Use of spreadsheets for calculating and graphing*
- *Data analysis, application of mathematical models, and parameter evaluation*

The multidisciplinary Engineering Clinic sequence at Rowan University is a required design course, taken during every 14-week semester from freshman to senior year, exposing students to real-world engineering problems at increasing levels of complexity with each year. At the freshman level, where this experiment was implemented and assessed, the students are introduced to the art of design, unifying engineering science principles, and the basics of experimental design via one three-hour lab per week.

LITERATURE REVIEW

Principles of rheology

Rheology is the study of the deformation and flow behaviors of fluids, with the prefix "rheo" referring to "something that flows" in Greek. The rheological behavior of fluids is often studied with the application of shear forces across the fluid. Shear force (F) occurs if a force is applied parallel to a plane of a surface,[7] in this case the x-direction (Figure 1).

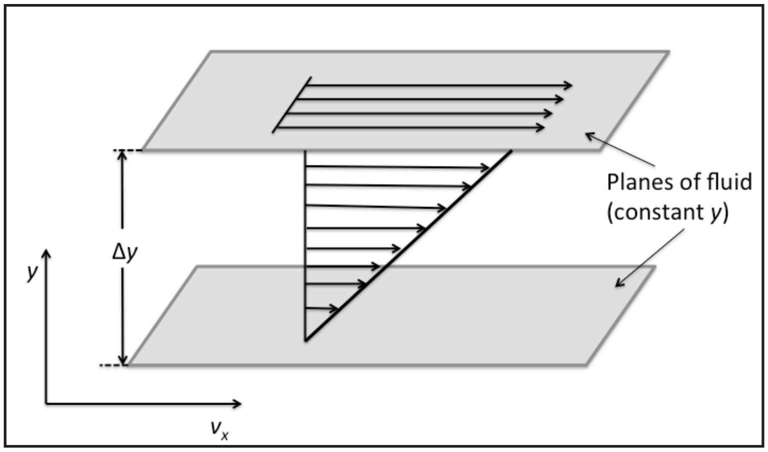


Figure 1. *Shear between layers of fluid.*

This results in shear deformation of the "layers" of fluid, since they will flow past each other at a velocity v_x. Shear stress is calculated by dividing shear force by the area over which it is applied. The application of shear stress will produce a velocity gradient among the layers of fluid in the y-direction, as shown in Figure 1, which can be expressed mathematically as dv_x/dy. This velocity gradient is also known as the shear rate.[8] To characterize the rheological behavior of the fluids, shear stress is plotted versus shear rate. The shape that this plot takes allows us to classify the fluid (Figure 2).

The most common type of fluid is called Newtonian, meaning that shear stress versus shear rate yields a straight line. The slope of this line is defined as the viscosity of the fluid. Viscosity can be considered as resistance to shear; the higher the viscosity of the fluid, the harder it is to shear it. A shear thinning fluid is one that decreases in viscosity as shear rate increases. A shear thickening fluid, quite intuitively, is the opposite of a shear thinning fluid, as its viscosity increases with shear rate. A Bingham plastic is similar to a Newtonian fluid in that its viscosity does not change with added shear rate, but it is different because it requires a minimum shear stress, known as the yield stress, in order to move.[8]

Rheology of healthy blood

Blood is a particular complex fluid, as its rheology can be thought of as a combination of a Bingham plastic and a shear thinning fluid in that it requires a yield stress to move, and its viscosity decreases with increasing shear rate.[9] Blood is a two-phase fluid: one phase is the plasma and the other the hematocrit. Plasma contains dissolved proteins, such as fibrinogen, salts, carbohydrates, and other compounds, and acts as a Newtonian fluid.[10] The hematocrit is the portion of the blood that consists of the cells, which include RBCs (95% by number), platelets (4.9%), and white blood cells (0.13%). Typically, human blood is approximately 55% by volume plasma and 45% hematocrit.[10]

Hematocrit content, RBC aggregation, and deformability are the key factors that regulate whole blood viscosity.[11-13] The presence of red blood cells causes blood to exhibit shear thinning behavior. The relatively high viscosity of blood at low shear rates occurs because of the formation of rouleaux, or aggregates of RBCs that inhibit flow.[14] As shear rate increases, the rouleaux are broken up, and thus the viscosity decreases.[10] In addition, healthy RBCs can deform under shear stress; for instance, they form a bullet shape during passage through small capillaries.[10] This deformation allows RBCs to partially accommodate shear stress[15] and orient

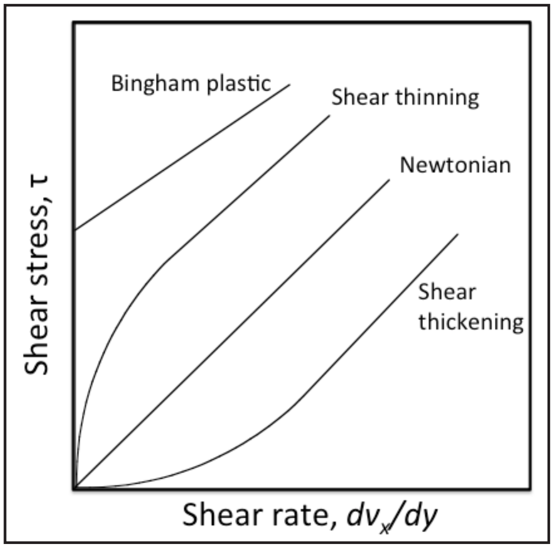


Figure 2. *Relationships between shear stress and shear rate for Newtonian and non-Newtonian fluids.*

themselves in the direction of the flowstream.[16] The formation of rouleaux and this deformability are the major factors contributing to the shear thinning behavior of blood at higher shear rates[17] Studies have shown that suspensions of rigid spheres do not shear thin like suspensions of deformable RBCs.[16] Finally, the viscosity of blood increases with hematocrit concentration.[9]

A number of constitutive equations have been proposed that describe the rheological properties of blood. A commonly used model is the Casson model,[10] given in Eq. (1):

$$\tau^{0.5} = a\gamma^{0.5} + b^{0.5} \tag{1}$$

where τ is the shear stress, Y is the shear rate, and b is the yield stress. The constants, a and b, are found by a linear regression of the square root shear stress versus the square root of shear rate. The values are dependent on plasma viscosity and composition as well as hematocrit content.[10]

Rheology of sickle cell blood

Sickle cell anemia is a disorder that alters the microrheology of RBCs and, in turn, the rheology of the whole blood. Dehydration and structural changes in the sickle RBC membrane causes them to become inflexible.[14] Thus, they do not deform at high shear rates, causing sickle cell anemia blood rheology to lack the shear thinning effect.[16] In addition, the presence of inflexible particles causes sickle cell blood to exhibit a higher viscosity than healthy blood.[18] Clinical consequences of sickle cell anemia include the increased work required of the heart to pump blood (more viscous blood), tissue infarction, and organ failure.[18] Tissue infarction is the death of tissue cells due to a lack of sufficient blood supply.

Chitosan

Chitosan, a natural biopolymer derived from crustacean shells, has been used in a number of biomedical applications, due to its biodegradability and biocompatibility, especially in the sustained delivery of drugs.[19-23] In this experiment, soft, hydrated chitosan particles are prepared as described previously[24] and are used to simulate healthy RBCs. Chitosan particles can also be easily crosslinked via a Schiff's base chemistry[25] with dialdehydes, such as glutaraldehyde. This reaction alters the polymer structure of the particles, lessening the water absorption capability and increasing rigidity. Thus, crosslinked particles are used to simulate sickle cells.

Pedagogical framework

A number of rheological experiments have been developed and published in the educational literature, most of them focused on polymers and applications within the chemical engineering discipline. These experiments were developed for the purposes of understanding and improving polymer processing techniques. For instance, Liu, *et. al.* from Eastern Illinois University developed an experiment involving a Laboratory Capillary Rheometer (LCR) that provides insight into injection molding and the changes in polymer structure that occur with processing.[26] At Kettering University, students use an LCR and On-Line Process Rheometer (OLPR) to study rheological properties of polymer melts.[27] At the University of Southern Maine, Marshall, *et. al.* describe an experiment on characterizing flow characteristics of magneto-rheological fluids, with technological applications to electromechanical and electrohydraulic equipment.[28]

Since the field of biomedical engineering has grown tremendously in recent years, educators have been focusing on developing new coursework to meet the industry demand for students who are well versed in both engineering and biology fundamentals. As such, there have been published reports on the applications of fluids to biological systems. At Vanderbilt, in a new course called Physiological Transport, students are given different biological scenarios related to the respiratory tract or cellular transport, and they must develop models describing the phenomena.[29] Merrill, *et. al.* described active-learning activities that teach students to computationally model transport processes such as a drug-eluting stent and therapeutic contact lenses for glaucoma.[30] There are fewer reports on hands-on experimental activities in biorheology. At Bucknell, students construct a recirculating flow system utilizing pumps, pressure gauges, tubing, fluid reservoirs, and various fittings. They measure pressure drop in a specific section of the system, analyze frictional losses, and relate the results to the circulatory system in the body.[31] In this paper, we address the need for more hands-on biomedical content in engineering curricula. We uniquely integrated concepts in transport phenomena and biomaterials science into a scalable and cost-effective laboratory experiment that is readily implementable into both core engineering curricula and biomedical engineering programs.

LABORATORY DESCRIPTION

In this experiment, the rheological behaviors of the following samples are studied with a rotational viscometer and the data is fit to the Casson model.

- *A single-phase, 80% aqueous glycerol solution simulates blood plasma.*
- *Chitosan particles in 80% aqueous glycerol create the healthy two-phase blood analog. The chitosan particles are suspended in a concentration of 25-75 mg/mL, replicating various hematocrit levels.*
- *Glutaraldehyde-crosslinked chitosan particles (25 mg/mL) in 80% aqueous glycerol create the two-phase sickle-cell blood analog.*

Materials

- *Chitosan (100 kDa, Acros Organics, Fairlawn, NJ)*
- *Acetic acid solution (2% vol)*
- *NaOH solution (5M)*
- *Glycerol, aqueous solution (80% wt)*
- *Glutaraldehyde, aqueous solution (25% wt)*

Equipment

- *Tabletop centrifuge*
- *Vacuum dryer*
- *Rotational viscometer*

Preparation of chitosan particles (healthy RBCs)

1. *Prepare a 1.25 % (wt) solution of chitosan in 2 % (vol) acetic acid.*
2. *Pour 25 mL of 5 M NaOH slowly into the chitosan solution. Stir gently to suspend chitosan particles as they precipitate.*
3. *Wash the mixture with DI water by centrifugation at 3200 RPM. Repeat five times to ensure removal of the NaOH.*
4. *Dry the particles under vacuum.*
5. *Once they are dry, crush the particles into a powder using a mortar and pestle.*
6. *Suspend the particles by vortexing the desired amount in 80% aqueous glycerol solution.*

Preparation of the glutaraldehyde-crosslinked chitosan particles (sickle cell RBCs)

1. *The chitosan particles are made more rigid by glutaraldehyde crosslinking. To do this, the particles are soaked in 25 % (wt) glutaraldehyde at 60°C for 8 hours while stirring gently to suspend the particles.*[24]
2. *The crosslinked particles are washed and dried under vacuum using the same procedure described above.*
3. *Suspend the particles by vortexing the desired amount in 80% aqueous glycerol solution.*

Viscosity measurement

To analyze the flow characteristics of the blood analogs, the Brookfield RVDVII+ Rotational Viscometer with a No.M06-084 UL Adapter is used to create a plot of shear stress versus shear rate. The shear rates and shear stresses are recorded after the readings become sufficiently stable. All viscosity tests are conducted at room temperature.

DATA ANALYSIS

A comparison of the viscosity of single-phase and two-phase blood analogs, with 25 mg/mL of suspended chitosan particles, is shown in Figure 3. The single-phase blood analog exhibits a viscosity that is more or less constant as a function of shear rate, thus it exhibits Newtonian behavior, consistent with what has been reported for blood plasma.[16] The two-phase healthy blood analog exhibits behavior similar to whole blood, where the viscosity is high at low shear rates, and then decreases with increasing shear and approaches a minimum value.[9, 32] Compared to this, the sickle cell blood analog exhibits markedly decreased shear thinning behavior. This is attributed to the fact that crosslinking enhances the rigidity of the chitosan particles. Like in sickle

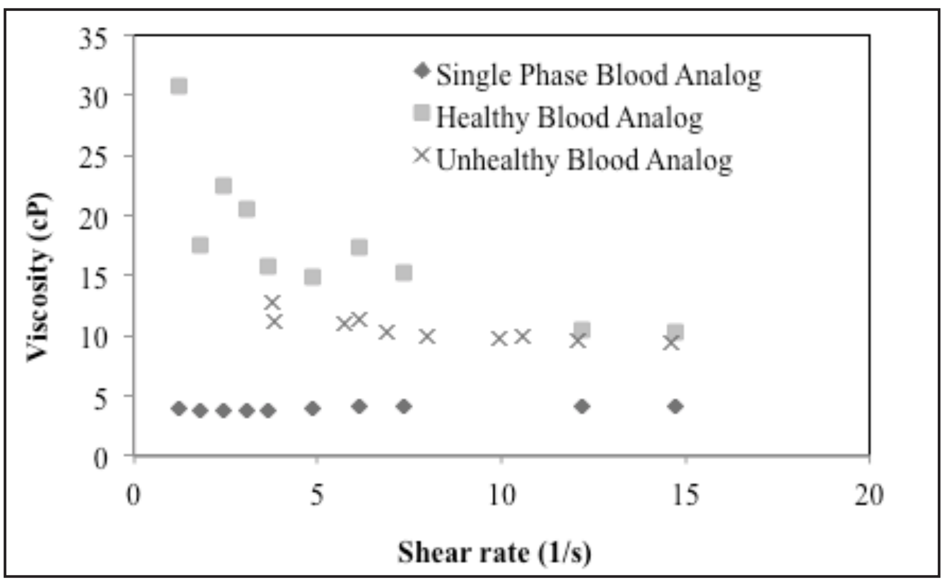


Figure 3. *Rheological comparison of single, healthy, and stiffened blood analogs.*

cell blood, this lack of deformability translates to a lessening of the shear thinning effect. These trends are consistent with what was found by Baskurt and Meiselan,[16] who used chemically stiffened whole blood.

The effect of varying hematocrit level (between 25 and 75 mg chitosan particles/mL) on the viscosity of the healthy two-phase blood analog is shown in Figure 4. The square root of shear rate versus shear stress is plotted in order to facilitate linear regression for the determination of the a and b parameters in the Casson model [Eq. (1)]. The good fit (R>0.99) of these regressions indicates that the samples' behavior is well characterized by the Casson Model. The Casson equation parameters are listed in Table 1. Together, Figure 4 and Table 1 show that the two-phase blood analogs exhibited increasing trends in viscosity as the concentration of chitosan particles in the suspension increased from 25 to 75 mg/mL. In previous studies, similar results were also found for blood samples of different hematocrit.[9]

TABLE 1
Casson Equation Constants For Varied Hematocrit Levels
The uncertainty is reported as 95% confidence intervals.

Hematocrit (g/mL)	a	b
0.0250	0.8546 ± 0.0072	0.0134 ± 0.0053
0.0375	0.9107 ± 0.0537	0.2898 ± 0.0978
0.0625	1.2234 ± 0.0194	0.3094 ± 0.0475
0.0750	1.3068 ± 0.0547	0.4313 ± 0.1429

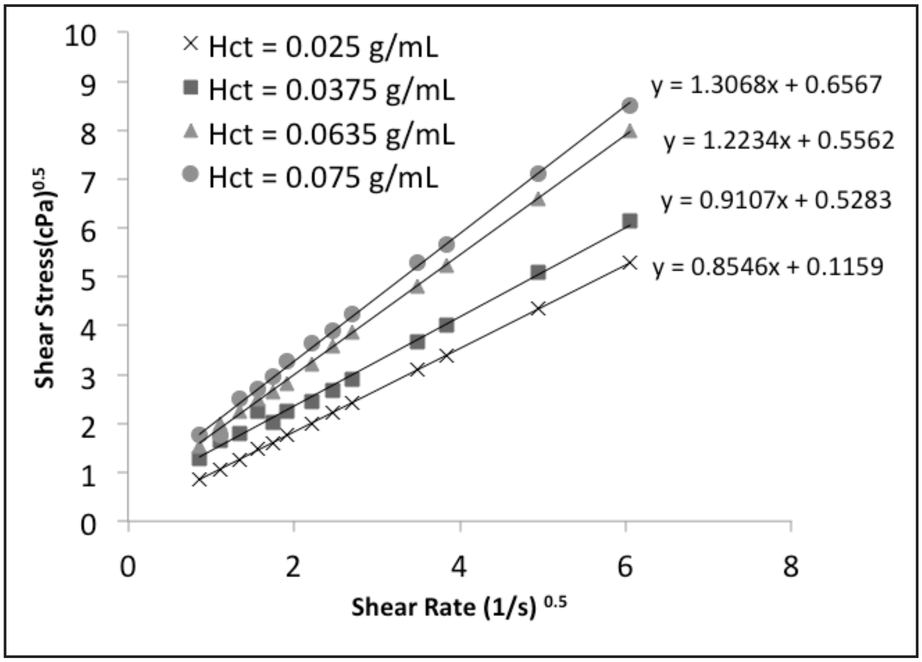


Figure 4. Linear regression analysis for varied hematocrit levels.

SUMMARY OF ASSESSMENT AND LEARNING

To evaluate the impact of this experiment on student learning, a quiz was administered to students before and after the lab. The quiz comprised seven multiple-choice questions that were mapped to lab objectives and ABET objectives. A summary of the quiz questions is provided in Table 2 (next page) in which multiple-choice questions are presented as correct statements for brevity. Questions were mapped to Rowan Engineering Clinic II course objectives and ABET outcomes as shown in Table 3 (page 155). The table also shows the measurable skills that are associated with each outcome.

Figure 5 shows the average score on the pre-test was 42%±9%, and the average score on the post-test was 92%±5%, for n=17 students. For ABET 3a/Rowan 1 and ABET 3b, the percentage of correct responses increased 48 and 59%, respectively,

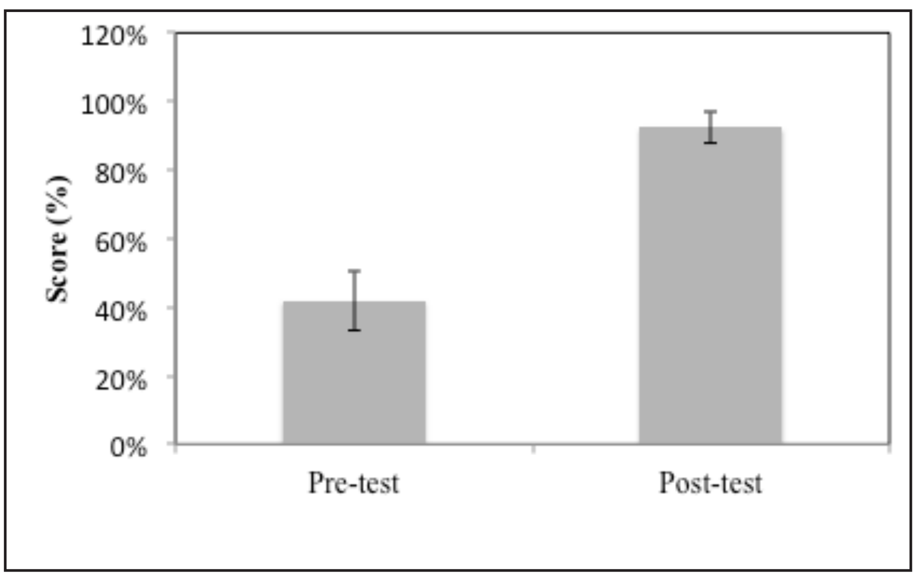
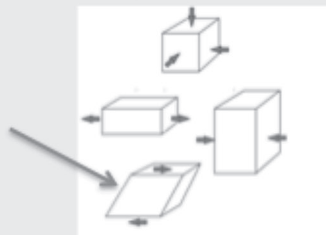
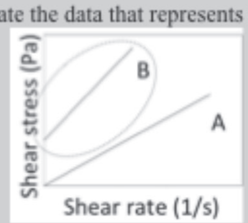


Figure 5. Average score on the pre- and post-tests (n=17).

TABLE 2

Pre- and Post-module Questions Presented as Correct Statements

The boldface text indicates the choice that correctly completes each statement.

Question #	Correct statement
1	Rheology is defined as **the study of how liquids and soft solids flow under a force.**
2	In the following, indicate the depiction that best represents a shear force. 
3	Shear stress is defined as **the application of force across a fluid.**
4	The units of shear strain are: unitless.
5	In the following figure, indicate the data that represents a fluid with a yield stress (line A or B?) 
6	In the above figure, line A represents a fluid where there is a linear relationship between shear rate and shear stress. This means the fluid is a **Newtonian fluid**.
7	Sickle cell anemia is a blood disorder where red blood cells become crescent-shaped and lose flexibility. This causes blood viscosity to **increase**.

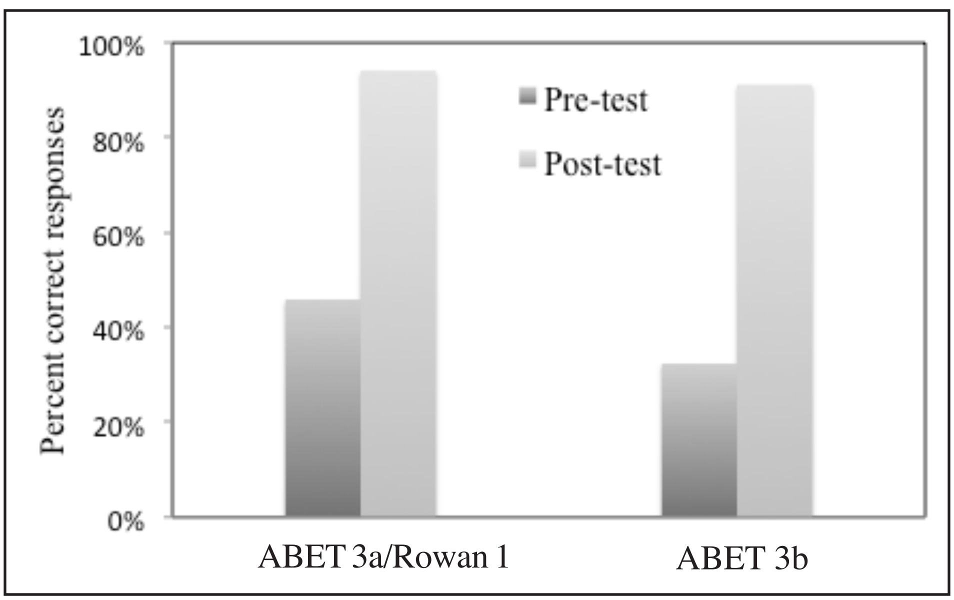


Figure 6. *Percentage of correct responses for each learning outcome as described in Table 3. The percentage includes responses for questions mapped to a particular outcome.*

between the pre-test and the post-test (Figure 6).

This activity, as it is designed, offers flexibility in the required time for completion. For instance, variables such as hematocrit content can be excluded. As an alternative, groups of students can study the different blood types (single-phase, two-phase healthy, sickle cell analog) or hematocrit contents in parallel during a single laboratory period. If the students have access to a cone and plate rheometer, rheological curves may be generated more quickly, the shear rate and temperature can be more precisely controlled, and sinusoidal deformation may even be applied. In this case, evaluation of the loss and storage moduli may give students greater insight into how the particle crosslinking affects the viscous and elastic-like properties of the blood analogs.

CONCLUSIONS

The rheology of blood is particularly complex because of the geometry of RBCs, the hydraulics of the vasculature in the body, and the cellular abnormalities of sickle cells. Here, we have extracted the basic principles and formed them into an experiment designed to introduce students to rheology. The experiment was implemented in a multidisciplinary Freshman Engineering course at Rowan University. Students created healthy and sickle cell blood analogs with safe and cost-effective chemical components—water, glycerol, chitosan, and glutaraldehyde. They explored flow curves for these materials as the hematocrit content and blood cell deformability was varied. The analysis of experimental data introduces students to spreadsheet calculations, data analysis, and mathematical modeling. Our evaluation indicates that the experiment contributed to outcomes set forth by Rowan University and ABET for undergraduate chemical engineering students.

ACKNOWLEDGMENTS

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TABLE 3
Pre- and Post-Module Assessment Questions Alignment to ABET and Rowan Standards For Undergraduate Chemical Engineering Students

Outcome	Measurable skills categorized within this outcome:	Pre- and post-test questions
An ability to apply knowledge of mathematics, science, and engineering (ABET 3a); Students demonstrate the ability to apply principles of organic, inorganic, materials chemistry, as well as some science topics at the advanced level (Rowan 1)	Students will define the field of rheology and Newtonian/non-Newtonian fluids; characterize relevant parameters describing fluid behavior.	1-4, 7
An ability to design and conduct experiments, as well as to analyze and interpret data (ABET 3b)	Experience with conducting a rheological characterization of various fluids with a rotational viscometer; categorize fluids based on shear stress-shear rate behavior; correlate results to fluid composition.	5-6

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