Effective and accurate methods for characterizing rheological changes of proteins by alteration of their level, composition, or conformation are of significant clinical and biotechnological utility. Current implementations to measure rheological properties of a fluid, however, require mechanically moving components or off-chip measurement setups, thereby preventing incorporation of them into microenvironments. Thus, it is challenging for the development of a simple and effective means for rheological measurement in microenvironments. We demonstrate a new class of rheometer that balances between a sample and reference fluid with a common pressure drop, thereby enabling simple and accurate measurement of fluid viscosity, and eliminating the use of standard pressure gauges and complicate theoretical calculations. We also demonstrate the robustness of the rheometer for practical applications by measuring minute changes in a rheological property of bovine serum albumin (BSA) by chemical and thermal denaturation.

Blood-plasma viscosity is determined by various macromolecules such as fibrinogen, immunoglobulin, lipoproteins, and albumin that serve many important functions in blood flow including supply of insoluble fibrin clot, identification of foreign objects, and transport of molecules with low water solubility. This important rheological parameter can be influenced by diseases and metal ions with alteration of protein level, composition, and conformation. The elevated viscosity significantly increases the risk of inflammatory disease, coronary heart disease, and stroke, and can result in rheologically impaired microcirculation, accompanied by red-blood-cell aggregation, platelet thrombogenesis, and vascular dysfunction. Therefore, effective and accurate methods for characterizing rheological changes of fluids containing molecules by alteration of their level, composition, or conformation are of significant clinical and biotechnological utility, and can facilitate the understanding of the relationship between blood rheological properties and diseases, and the physiological effect of the properties on blood vessels.

A rheometer is a device to measure the way in which a liquid flows with varying flow conditions, and can be categorized into two groups according to applied stress: shear and extensional rheometers. Despite their successful demonstration, most of rheometers utilize mechanically moving components such as a rotating cylinder or plate to apply stress to a liquid, thereby preventing incorporation of them into microenvironments and direct observation of the environment. Although shear rheometers with miniaturized sliding plates have been developed to measure the steady shear viscosity, the high-precision alignment between two parallel plates makes it difficult to fabricate and operate them. Capillary viscometers force a liquid through a tube of constant cross section, and measure the flow rate of the liquid and pressure drop between two channel ends, thereby enabling easy miniaturization without mechanical components. However, the main drawback of these methods is that the accuracy of the measured viscosity values is highly dependent on an external pressure transducer for measurement of the pressure drop. Due to channel-entrance and -exit effects, it is difficult to accurately measure the pressure drop between microchannel ends. In addition, the complicated theoretical modeling and calculation for a non-Newtonian fluid make it difficult to measure its viscosity in microchannels with continuously varying cross sections that mimic a geometric characteristic of blood vessels. It thus appears that there is a need for a simple and effective method to measure the rheological properties of a fluid without any measurement setup such as standard pressure gauges and limitation of channel shapes to be used for rheometers.

To address the above need, we present here a simple and effective method to measure a rheological property of a fluid using a comparator channel design that consists of two microchannels connected in parallel. Figure 1 shows the comparator design and the principle of measuring an unknown viscosity without any measurement setup. A microfluidic comparator has been demonstrated to measure the variation of the pressure drop by cells and droplets passing a microchannel. Since its channel circuit is not in real parallel, it is impossible to balance two different channels with a common pressure drop. It also requires calibration of the interface deflection of the indicating fluid as a function of the change in pressure drop. In contrast, the operational principle of the microfluidic rheometer resembles a...
Wheatstone bridge. From the electronic-hydraulic analogy, the laminar incompressible flow of steady state inside a channel is described by

\[ Q = \frac{\Delta P}{R} \]  

where \( Q \) is the volumetric flow rate, \( \Delta P \) is the pressure drop from one end of the channel to the other, and \( R \) is the hydraulic resistance of the channel.\(^{[10]}\) The hydraulic resistance of a channel of uniform cross section can be defined as\(^{[11]}\)

\[ R = C(\alpha) \frac{\mu L}{2D_h A} \]  

where \( C \) is the product of the Darcy friction factor and Reynolds number that is a constant at the fixed aspect ratio (\( \alpha \)) of the channel, \( \mu \) is the viscosity, \( L \) is the channel length, and \( A \) is the cross-sectional area of the channel. \( D_h \) is the hydraulic diameter, defined as \( D_h = 2wh/(w + h) \), where \( w \) and \( h \) are the width and height of the channel, respectively. \( \mu_x \) is the unknown viscosity to be measured; \( \mu_{\text{ref}} \) is known as a reference viscosity, and the flow rates \( Q_x \) and \( Q_{\text{ref}} \) are adjustable (Figure 1a). The volumetric flow rates through the channels are adjusted until two marked flows separately branch into two parallel channels, thereby providing information concerning the volumetric flow rate through each channel (Figure 1b). At the point of balance, Equation (1) is applied separately for each channel, and because the pressure drop and channel dimensions are the same for both parallel channels, we equate both expressions to yield

\[ \mu_x = \mu_{\text{ref}} \frac{Q_{\text{ref}}}{Q_x} \]  

where subscripts \( x \) and \( \text{ref} \) denote fluids of unknown and known viscosity, respectively. The balancing between two different fluids with a common pressure drop enables simple and accurate measurement of fluid viscosity, and eliminates the use of standard pressure gauges and complicated theoretical calculations. We used this microfluidic principle to measure the microrheological property of a globular protein model system, BSA.

The microfluidic rheometer is first characterized with sucrose solutions of known viscosity for calibration. The applied flow rate of sucrose solutions was varied from 1 to 5 \( \mu \text{L min}^{-1} \), which corresponds to the shear rate from 134 to 672 \( \text{s}^{-1} \).

In this range of shear rate, the sucrose solution behaved as a Newtonian fluid being independent of shear rate and stress. Figure 2 shows the concentration-dependent increase in the viscosity of sucrose solution. In the case of liquids, the viscous forces are due more to the breaking of cohesive forces than to momentum transfer because molecules are close together and their velocities are relatively lower than gas.\(^{[12]}\) Therefore, high molecular concentration can increase the fluid viscosity by increasing the closer proximity of molecules in motion and enhancing the opportunity for momentum exchange. For comparison, the viscosity data of sucrose solution from literature is included in Figure 2 (dashed line) and can be defined as\(^{[13]}\)

\[ \eta = 6581/[(61.5 + \theta) - (1 + 0.0110\theta)c]^2 \]  

where \( \eta \) is the viscosity in centipoise (cP), \( \theta \) is the temperature \( ^\circ \text{C} \), and \( c \) is the sucrose concentration in g 100 mL\(^{-1} \) (%). The measurement results obtained via the microfluidic rheometer closely overlay the data from literature.\(^{[13]}\)

The sigmoidal plot for unfolding of BSA shown in Figure 3a strongly suggests that the rheometer is able to differentiate the conformational change of proteins in microflows. Concentrated urea was added to BSA solutions to achieve a desired urea
The unfolding of proteins induces the increase of their protein absorption on the measurement results is negligible. The unfolding midpoints (unfolded fraction \(1/2\)) of 5.9 and 6.4M from the literature.[15] Literature results based on dynamic light scattering (DLS) results suggested that BSA during urea denaturation existed at three different conformations; native, intermediate, and unfolded.[16] The intermediate state can be characterized by a natively folded state at 3M urea, thereby showing the weaker dependence on urea concentration in the conformation change of BSA. Similarly, we observed that the viscosity increase during urea denaturation has a transition point at 4.1M (see the inset plot of Figure 3a). In the range of urea concentration below the transition point, the viscosity increase has the weaker concentration dependence. For thermodynamic analysis of the unfolding curve, the fractions of the native and unfolded states of BSA are evaluated assuming a two-state model, described as follows:

\[
K_{eq} = \frac{n_{n} - n_{y}}{n_{y} - n_{u}}
\]  

(6)

\[
\Delta G = -RT \ln K_{eq}
\]  

(7)

where \(K_{eq}\) is the equilibrium constant; subscripts \(n, u,\) and \(y\) denote native, unfolded states, and somewhere between both states, respectively; \(G\) is the Gibbs free energy, \(R\) is the gas constant, and \(T\) is the absolute temperature. It can be seen in Figure 3b that \(\Delta G\) varies linearly in the limited region of denature concentration where \(\Delta G\) can be measured. The analysis of BSA unfolding gives a Gibbs free energy of 13.1 ± 1.1 kJ mol\(^{-1}\), which presents the conformational stability in the absence of urea (Figure 3b). The \(\Delta G\) measured are comparable to the data from the literature of 15.5 ± 3.2 kJ mol\(^{-1}\) determined from the hard-sphere rheology hypothesis.[15] These results support that the microfluidic rheometer well reflects the change of viscosity by conformational alteration of BSA with a minimum measurable difference of \(\approx 0.06\) cP.

The unfolding state of BSA in urea possesses a randomly coiled conformation via the irreversible denaturation process that neither aggregates nor adsorbs significantly[17] and shows the dependence on urea concentration as shown in Figure 3a. On the other hand, the thermal denaturation results in a molten globule state and soluble aggregate that are formed through

momentum exchange. The relationship between the molecular size of a protein and the viscosity of protein suspension can be expressed with the hard-sphere theory[14]

\[
\eta = \eta_{s}(1 + 2.5V + 6.2V^2)
\]  

(5)

where \(V\) is the equivalent spherical volume fraction occupied by the protein and \(\eta_{s}\) is the solute-free viscosity. From this equation, the hydrodynamic diameter for any protein can be derived, and for BSA this equation yields hydrodynamic radii of 44.3 ± 1.0 and 62.7 ± 0.9 Å for the native and unfolded state, respectively. The molecular sizes calculated are comparable to the data from the literature of 34.2 ± 1.4 and 75.3 ± 1.1 Å for the native and unfolded state, respectively.[15] The unfolding midpoint (unfolded fraction \(= 0.5\)) of 5.6M that we measured is also comparable to the midpoints of 5.9 and 6.4M from the literature.[15]
intermolecular association of BSA molecules such as the hydrogen bond of beta-sheets between monomers.\[^{17}\] The structural change is also reversible only below the critical temperature of \(\approx 65^\circ\text{C}\).\[^{18}\] With the rheometer, we observed such threshold response in the rheological viewpoint with an increase of temperature (Figure 4). The heat-treated samples with a constant BSA concentration of 0.75 mM were injected into the rheometer at flow rates ranging from 1 to 16 \(\mu\text{L}\text{ min}^{-1}\), which correspond to shear rates from 134 to 2150 \(\text{s}^{-1}\). In this range of shear rate, the BSA solutions behaved as a Newtonian fluid. When BSA samples are heated to 50 \(^\circ\text{C}\) and cooled down, there is no significant difference in the rheological aspect (Figure 4). The heat-treated BSA samples still maintain the rheological property in their native state, which reflects the refolding of BSA closer to its native state. Heating up to more than 65 \(^\circ\text{C}\) produces the irreversible change in the rheological property that is temperature- and time-dependent. In colloid science, two limiting cases are usually considered: perikinetic aggregation, where the particle encounters are due to Brownian motion, and orthokinetic aggregation, where the collisions are caused due to the gradient velocity field. The ratio of the aggregation rates for orthokinetic \(J_o\) over perikinetic aggregation \(J_p\) is given by\[^{19}\]

\[
\frac{J_o}{J_p} = \frac{sd^3\psi\eta}{2\pi k_B T} \quad (8)
\]

The calculated ratio is \(2.7 \times 10^{-4}\) for the effective swelling factor due to unfolding, \(s = 5\); the molecular diameter, \(d = 6.3 \times 10^{-9}\) m; the shear rate, \(\psi = 2150\text{ s}^{-1}\); the Boltzmann constant, \(k_B = 1.38 \times 10^{-23}\) J K\(^{-1}\); and the temperature, \(T = 303\) K. This value means that applying a shear field should not significantly enhance the aggregation rate and orthokinetic aggregation can be negligible in the measurement procedure.

In conclusion, we have demonstrated a simple, effective method to measure a rheological property and its change of protein in microenvironments with a minimum measurable difference of \(\approx 0.06\) cP. Additionally, this work demonstrates a solution to the challenging problem of rheological measurement without precise measurement setups such as standard pressure gauges and limitation of channel shapes to be used for rheometers. The proposed rheometer uses two conventional syringe pumps to apply stress to a liquid. This operation scheme can be easily replaced with miniaturized pumps and other devices to pressurize a liquid. While we demonstrated the unfolding and aggregation of BSA in the experiments reported here, this new class of rheometer with its simplicity and efficient capability for measurement can be further used to unveil the relationship between blood rheological properties and diseases, and the physiological effect of the properties on blood vessels.

**Experimental Section**

**Design and fabrication of microfluidic rheometer:** The microfluidic rheometer was fabricated in poly(dimethylsiloxane) (PDMS) by soft lithography, as shown in Figure 1b, in which the width of the linear channels in parallel connection was 700 \(\mu\text{m}\) and the length was 4.2 mm. A mold containing the comparator channel designs was obtained by patterning an SU-8 photoresist (Microchem Corp., MA) on a silicon wafer by standard photolithography. Liquid PDMS mixed with the curing agent (ratio of 10:1) was cast on the mold and cured for 3 h in a convection oven at 65 \(^\circ\text{C}\) for complete crosslinking. The PDMS channel was sealed with a glass slide after exposure to oxygen plasma for 30 s. The channel dimensions were measured with a surface profiler (Alpha-step 500; KLA tencor Corp., CA); the height was 32.6 \(\pm 0.4\) \(\mu\text{m}\).

**Material preparation:** All aqueous solutions were prepared with deionized (DI) water from the Milli-Q filtration system (Millipore Co., MA). BSA (30036-578, Fraction V, IgG free, fatty acid free) was purchased from Invitrogen (Carlsbad, CA). Sucrose (179949) and urea (U5378) were obtained from Sigma-Aldrich (St. Louis, MO). A red food dye (Oh Jung Commercial Co., Korea) was dissolved in DI water with a concentration of 8 mg mL\(^{-1}\). The dye solution with a viscosity of 0.86 cP at 30 \(^\circ\text{C}\) was used as a reference solution for viscosity measurement. Water at 30 \(^\circ\text{C}\) has a viscosity of 0.79 cP.\[^{13}\]

**Experimental setup:** All viscosity measurements were conducted at 30 \(^\circ\text{C}\) in an incubator system for cell culture (Live Cell Instrument, Korea) and repeated more than three times. The microchannels were imaged with a microscopy (Zeiss Axiovert 25; Carl Zeiss, Germany). To induce thermal unfolding and aggregation of BSA, the protein solutions were heated for a defined period of time in a heating block (Thermomixer Compact; Eppendorf, Germany) at 300 rpm, and then cooled down at 30 \(^\circ\text{C}\) in a water bath. Two syringe pumps (702212; Harvard Apparatus, Holliston, MA) with pumping accuracy of \(\pm 0.5\%\) and reproducibility of \(\pm 0.1\%\) were used to produce 1.0 to 21.9 \(\mu\text{L}\text{ min}^{-1}\) flows through the microchannels. Under this operational condition (low Re below \(\approx 1\)), no turbulent phenomena were observed at the entrance and exit of the channels. The apparent shear rate in a rectangular channel of \(w \times h\) can be calculated as\[^{20}\]

\[
\psi = \frac{6Q}{wh^2} \quad (9)
\]
Keywords:
microfluidics · protein aggregation · protein unfolding · rheometers


Received: February 9, 2010
Published online: May 11, 2010