Automatic Flow Analysis for Blood with Anticoagulant Using a Newly Developed Compact-Sized Falling Needle Rheometer

Hideki Yamamoto, Takamasa Suzuki
Department of Chemical, Energy and Environment Engineering, Faculty of Environmental and Urban Engineering, Kansai University, 3-3-35, Yamate, Suita, 564-8680, Osaka, Japan
e-mail: vyhideki@kansai-u.ac.jp

Kimito Kawamura
Department of Process Engineering Technology, Research & Development Laboratories for Sustainable Value Creation, Asahi Breweries, Ltd, 1-1-21,midori, Moriya, 302-0106, Ibaraki, Japan

Roberto Plasenzotti, Dominik Bernitzky
Core Unit for Biomedical Research, Medical University of Vienna, Waehringer Guertel 18-20 / AKH1Q, A-1090 Wien, Austria

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Abstract

A compact-sized falling needle rheometer with quick operation and automatic flow analysis has been developed for viscometry of anticoagulated blood. The volume of a sample of blood only needs to be 4 mL and the measuring time is within 3 min. Measured flow properties of human blood and rabbit blood with anticoagulant are evaluated as a flow curve showing the relationship between the shear stress and shear rate. The accuracy and the reproducibility of the presented rheometer are ascertained by viscosity measurements of standard liquid for calibration of viscometers manufactured by Nippon Grease Co., Ltd.. The standard liquids of JS10 and JS20 at 310.15K were chosen after careful consideration of the blood viscosity range (3.0 mPa•s to 10.0 mPa•s). Good uncertainty within 0.5% and reproducibility within ±1.0% are confirmed by comparison with reference data of standard liquids. Observed flow curves of the human and rabbit bloods with anticoagulant show three typical fluid regions, these are, the Non-newtonian fluid region for a low shear rate range of 0<γ<200 s⁻¹, the transition region for the range about 150<γ<200 s⁻¹ and the Newtonian fluid region for a high shear rate range of about 200 s⁻¹<γ. It is found that the range of blood apparent viscosities are 4.5 to 6.5 mPa•s for human blood and 3.5 to 4.5 mPa•s for rabbit blood. This paper is concerned with the flow analysis of fresh human blood viscosity without anticoagulant using a newly developed compact-sized falling needle rheometer.

1 Introduction

The rheological properties of the blood is not only one of the important factors in the pathological diagnosis of the human body, but also basic data essential for analytical study of the change of fluid mechanics of the blood arising from the deterioration of the health condition. The rheological properties of blood are also important factors in pathological nexuses, but basic data of the viscosity of anticoagulated blood, especially non anticoagulated blood, are still lacking. In this context, the development of viscometry with high accuracy and quick operation, as well as the establishment of a data evaluation method by pathology are largely required. However, currently, there is little observed data of blood viscosity from measurement immediately after collection of a blood sample in comparison with the viscosity data of blood added with anticoagulant. [1-4] In research so far, it was found that the flow properties of human blood are available for preventive medicine for blood dyscrasia, clinical medicine, health care, functional foods, or the inspection of the effects of medicines. Also, it was reported that the viscosity of human blood influenced the concentration of fibrinogen in the plasma and the hematocrit value, and that the viscosity of human blood could offer important information for myocardial infarction and cerebral infarction, etc. However, the measurement of the viscosity just after blood collection from a body is not so easy, and the accumulation of numerical data of the viscosity is not yet sufficient. Most of the measurements of blood viscosity were carried out using blood added with anticoagulant, and at present there is little measurement of blood without anticoagulant. Also, it is problematical that many different viscometers are applied to these purposes, and a standard method has not yet been determined; hence, the values differ according to the type of device. The difficulties of flow analysis of blood come not only from an aggregation-dispersion phenomenon of red corpuscle cells, but also its transformation property,
many interaction forces between corpuscle cells and blood plasma. Therefore, the establishment of exact viscometry for fresh blood is desired for further discussion about the relationship between blood disease and its flow properties.

In this paper, a compact-sized falling needle rheometer and a flow analysis method using this new device for fresh blood with anticoagulant have been developed, and the relationship between the apparent viscosity and physical properties of fresh blood has also been evaluated.

The theory of the presented viscometer is mainly based on the Stokes type of equation, and this is a kind of a falling body viscometer [5-9]. The viscosity of human blood can be measured with a small blood sample of about 3.5 cm³ (total capacity is 4 cm³) and with rapid operation within 2 min after taking a blood sample from the human body. The total scale of this compact-sized falling needle rheometer is downsized to about 1/30 the size of the previous apparatus [6]. A circular cylinder needle made of polypropylene is applied for the experiment, and its outer diameter and total length are 2 mm and 20 mm, respectively. This needle is also minimized at 1/5 that of a previous needle [6]. The density of the falling needle is controlled by the mass of a sinker enclosed in the needle tube. Flow analysis of the sample fluid is carried out using the needle’s terminal velocity and the density difference between that of human blood and of a falling needle [10,11].

As stated above, the compact-sized falling needle rheometer is applied to the viscosity measurement of fresh blood before its coagulation. This paper is concerned with the development of a new rheometer for the measurement of flow properties of rabbit and human bloods.

![Photograph of the compact-sized falling needle rheometer for measurement of fresh blood viscosity](image1.png)

**Fig. 1** Photograph of the compact-sized falling needle rheometer for measurement of fresh blood viscosity

**Fig. 2** Schematic diagram of the compact-sized falling needle rheometer for measurement of human blood viscosity

### 2 Compact-Sized Falling Needle Rheometer with Automatic Operation

A schematic photograph of the compact-sized falling needle rheometer with automatic operation for measurement of blood viscosity is shown in Fig. 1. The schematic diagram of falling needle rheometer is illustrated in Fig. 2. The experimental apparatus consists of vertical double cylindrical vessels (one is a fluid vessel and the other is an insulating vessel cover) made of acrylic material (Fig. 2). The cap and bottom of the inner fluid vessel are made of Teflon. The inner fluid vessel for a blood sample is covered with the insulating vessel cover. The temperature of the inner fluid vessel is controlled at 310.15 K using a constant temperature air circulation by Peltier effect system within an uncertainty of 0.5 K. Constant temperature air is circulated in the space between the fluid vessel in the insulating cover. The diameter of the inner fluid vessel is 8.0 mm, and the height of the vessel is 90 mm. The total volume of the inner fluid vessel is about 4cm³. A needle collector for the collection of the falling needles is connected to the bottom of the inner fluid vessel via a needle-fluid separator made of Teflon. The needle-fluid separator is a slender cylindrical tube, and its diameter is 2.2 mm, which is similar to the needle diameter (2 mm) shown in Fig. 2.
When a sample fluid (human or rabbit blood) is introduced into the fluid vessel, the sample fluid does not leak into the space of the needle collector because the pressure in the needle collector is controlled at atmospheric pressure. After the operation of the first needle dropping automatically using needle falling system, the needle stopped at the bottom of the fluid vessel shown in Fig. 2 and is rapidly manually moved into the space in the needle collector by the guidance of a small magnet from outside the vessel. This movement of the falling needle from the bottom of the fluid vessel to the needle collector is important and indispensable for rapid measurement of fresh blood within 2 min. It was found in the preparatory experiment that the leakage of sample fluid into the needle collector from the fluid vessel is very little.

As each of the parts of the experimental apparatus such as the fluid vessel, the needle collector, the insulating vessel cover and a vessel stand shown in Fig. 2 can be taken apart easily, the collection of falling needles after the experiment can be very rapid and easy. This experimental apparatus is considerably compact for measurement of the viscosity of human blood compared with previous apparatuses.

<table>
<thead>
<tr>
<th>Needle No.</th>
<th>Density ((10^4 \text{kg}\cdot\text{m}^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.090</td>
</tr>
<tr>
<td>2</td>
<td>1.118</td>
</tr>
<tr>
<td>3</td>
<td>1.212</td>
</tr>
<tr>
<td>4</td>
<td>1.278</td>
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<tr>
<td>5</td>
<td>1.360</td>
</tr>
<tr>
<td>6</td>
<td>1.400</td>
</tr>
<tr>
<td>7</td>
<td>1.438</td>
</tr>
<tr>
<td>8</td>
<td>1.563</td>
</tr>
</tbody>
</table>

Table 1 Densities of falling needles used for viscometry of human blood and rabbit blood

The details of the falling needle used in this experiment is also given in Fig. 2(a) and (b). This falling needle is a slender hollow cylindrical tube made of polypropylene. The diameter of the needle is 2 mm and its total length is 20 mm. The shape of both sides of each needle is hemispherical. Eight falling needles with different densities shown in Table 1 are used in this experiment. The density of each falling needle is controlled by the mass of a sinker (iron) enclosed inside the needle tube. This sinker is fixed at the bottom of the needle tube so that the center of gravity is at a lower position. The density is calculated by the volume and mass of each needle, and its uncertainty is estimated to be within \(\pm 0.5 \times 10^{-3} \text{ g}\cdot\text{cm}^3\). Table 1 shows measured needle densities used in the experiment, they are determined in consideration of average blood density \((1.000 \text{ g}\cdot\text{cm}^3\) to \(1.100 \text{ g}\cdot\text{cm}^3\)).

In order to lead the falling needle to the center of the sample fluid, a needle inlet and needle launcher is equipped at the top of the fluid vessel. The needle launcher is a slender cylindrical tube as shown in Fig. 2. A pair of magnetic sensors is also installed at the middle part of the fluid vessel as shown in Fig. 2. The distance between magnetic sensors is 10 mm vertically. The passing time of each falling needle between magnetic sensors is automatically measured by a programmable controller manufactured by Sumitomo Metal Co., LTD. This magnetic sensor unit can be applied not only to clear liquids but also to opaque liquids. The programmable controller is connected to the personal computer via an amplification unit. It is possible to evaluate the falling velocity of each needle, and flow analysis such as a flow curve, apparent viscosity, and yield stress of the sample fluid can be measured automatically.

3 Experimental Method

Just after taking a blood sample from a vein, the blood (human blood or rabbit blood) is introduced into the fluid vessel shown in Fig. 2 and the top of the fluid vessel is covered with the Teflon cap in which the needle launcher is installed. This fluid vessel is equipped in the insulating vessel cover with two magnetic sensors. The vertical distance between the two magnetic sensors is 10 mm, and the fluid vessel is placed vertically on the vessel stand using a water level. Constant temperature air is circulated through the space between the fluid vessel and insulating vessel cover. The temperature of the fluid sample is kept at 310.15 K within an uncertainty of 0.5 K. Eight needles with different densities as shown in Table 1 are dropped down vertically in the sample fluid automatically using mechanical operation. The flow analysis is carried out using the observed passing time (terminal velocity) of the falling needles, needle densities, and blood density. Eight falling needles with different densities are used for measurement of the viscosity. Densities of blood are measured by the portable density/specific gravity meter (Kyoto Electronic Manufacturing Co., Ltd.) within an uncertainty of \(10^{-3} \text{ g}\cdot\text{cm}^3\). The calibration of the presented falling needle viscometer is carried out using a standard liquid for calibrating a viscometers (JS5, JS10) manufactured by Nippon Grease Co., Ltd. EDTA-2Na manufactured by Wako Pure Chemicals Co. Ltd., is used as an anticoagulant for both bloods.

4 Fluid Analysis using Compact-Sized Falling Needle Rheometer

Figure 3a and 3b show the model for flow analysis and velocity distribution in the compact-sized falling needle rheometer. This model is based on the flow analysis around the falling circular cylinder (falling needle) in the static fluid introduced into the cylindrical vessel. In order to apply this model to the motion of a falling needle and the mass transfer, the following four conditions are assumed [12].
Newtonian and non-Newtonian fluids are applied for the terminal velocity ($U_t$ in Fig. 3a). This model shows that the falling needle falls at a terminal velocity in the static sample fluid, the momentums affected on four surfaces of the minute circular cylinder core shown in Fig. 3a are balanced with each other, and they are balanced while the needle is falling at the terminal velocity. Therefore, this force balance can be described by the following equation:

$$\begin{align*}
P_1 \left( r + d \right)^2 \pi - r^2 \pi \right) + 2 \pi L \tau =
\end{align*}$$

$$\begin{align*}
P_2 \left( r + d \right)^2 \pi - r^2 \pi \right) + 2 \pi \left( r + d \right) L (\tau + d \tau)
\end{align*}$$

(1)

When $\Delta P = P_1 - P_2$ is less than 0, Eq.1 is arranged as follows:

$$\frac{1}{r} \frac{d}{dr} \left( r \Delta P \right) = \frac{\Delta P}{L}$$

(2)

Furthermore, while the needle falls at the terminal velocity in the sample fluid, the force balance of gravity, buoyancy, pressure and shear stress affected on the needle surfaces are given as

$$\left( \rho_s - \rho_l \right) g \pi (kR)^2 L + \pi (kR)^2 \Delta P = 2 \pi kR L \tau$$

(3)

In this equation, $\rho_l$ and $\rho_s$ are the fluid and needle density, respectively. The left-hand side first term of Eq.3 is the force of gravity and buoyancy, and the second term is the force of the pressure difference. The right-hand side term is the shear stress. This balance can be simply described by

$$\left( \rho_s - \rho_l \right) g + \frac{\Delta P}{L} = \frac{2 \pi kR L \tau}{kR}$$

(4)

Figure 3b illustrates the velocity distribution of the sample fluid due to falling of the needle. The amount of fluid (Q) to transfer between the falling needle surface and the container wall due to falling of the needle can be calculated by

$$Q = 2 \pi \int_r \rho \pi d \tau = \pi (kR)^2 U_t$$

(5)

Figure 3b shows that the sample fluid around the falling needle is pulled downward with falling of the needle in the static sample fluid. On the other hand, the fluid near the container wall rises with the falling needle. The
maximum velocity in the sample fluid is that on the needle surface. The maximum velocity is equal to that of the falling needle velocity. On the other hand, the velocity on the container wall becomes zero according to the above assumptions. Therefore, the boundary conditions of the velocity distribution can be described by

\[ u_{(r=R)} = -U_1 \]  
\[ u_{(r=0)} = 0 \]

In order to obtain the relationship between the shear rate and shear stress for the sample fluid, the Eqs. 2, 4, 5, 6a, and 6b and a constitution equation of the sample fluid are used simultaneously for flow analysis [6].

The constitution equation for a Newtonian fluid based on the law of viscosity is given by

\[ \tau = \mu \left( \frac{du}{dr} \right) = \mu \gamma \]  

where \( \mu \) is the viscosity, \( \tau \) is the shear stress, and \( \gamma \) is the shear state. The viscosity of the fluid sample can be calculated by the following equation from combining Eqs. 2, 4, 5, 6a, 6b, and 7.

\[ \mu = \frac{(\rho_1 - \rho_2)g(kR)^2}{2(k^2 + 1) \ln (k + 1 - k^2)} \]  

As \( R \) and \( k \) in Eq. 8 are fixed values according to the size of the fluid vessel and falling needle, they are arranged by the following equation using the geometric constant \( G \):

\[ G = \frac{2(k^2 + 1)}{(kR)^2 \ln (k^2 + 1) \ln (k + 1 - k^2)} \]  

Therefore, the viscosity of a fluid can be simply described by

\[ \mu = \frac{(\rho_1 - \rho_2)G}{GU_1} \]  

The shear rate of the sample fluid on the falling needle surface can be obtained from Eqs. 7 and 9 as

\[ \gamma_{(r=R)} = \frac{du}{dr} = \frac{(k^2 - 1)U_1}{kR(k^2 + 1) \ln (k + 1 - k^2)} \]  

The shear rate distribution for the radius direction can also be calculated by this equation. The shear stress of the sample fluid on the falling needle surface can also be described by the following equation by substituting Eqs. 8 and 11 for Eq. 7:

\[ \tau_{(r=R)} = \frac{(\rho_1 - \rho_2)(1 - k^2)kR}{2(k^2 + 1)} \]  

Equations 11 and 12 become fundamental data for flow analysis of the sample fluid. In this experiment, eight needles with different densities are used for the flow analysis. A flow curve of the sample fluid is obtained from the relationship the between shear stress and shear rate for eight needles [6].

5 Results and Discussion

5.1 Accuracy of the Compact-Sized Falling Needle Rheometer

The accuracy and the reproducibility of the presented rheometer are ascertained by viscosity measurements of the standard liquid for calibration of viscometers manufactured by Nippon Grease Co., Ltd. The standard liquids of JS10, and JS20 were chosen after careful consideration of the blood viscosity range (3.0 mPa•s to 7.0 mPa•s). Flow curves of standard liquid show in Fig. 5 and comparison of measured values with standard value are given in Table 2. Good uncertainty within 0.5 % and reproducibility within ±1.0 % are confirmed by comparison with reference data (Japanese Standard; JS10, JS20), and this experimental apparatus is improved from previous work (previous uncertainty was 1.25 %) [6]. It is thought that this improvement of accuracy was caused by the manufacturing method of falling needles, that is, the presented needle is manufactured using a model made of metal with high accuracy.

![Fig. 5 Flow curve of Standard liquids of JS10 and JS20 at 310.15K](image)

<table>
<thead>
<tr>
<th>Standard liquid</th>
<th>Viscosity [mPa•s]</th>
<th>This work</th>
<th>Standard value</th>
<th>Deviation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>JS10</td>
<td>4.987</td>
<td>4.982</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>JS20</td>
<td>9.006</td>
<td>8.963</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>
5.2 Flow Analysis of Human and Rabbit Bloods

Flow analysis of fresh human blood for female and male was carried out using a compact-sized falling needle rheometer using anticoagulant. Each result was evaluated as a flow curve, an apparent viscosity and hematocrit values. These rheological properties were compared between female and male blood.

In the experiment, fresh human blood of 20 cm$^3$ was taken from human veins, about 4 cm$^3$ of whole blood was rapidly introduced into the fluid vessel without anticoagulant. The temperature of the fresh human blood was kept at 310.15 K using a constant temperature water bath. The anticoagulant (EDTA-2Na) was added to the other blood (17 cm$^3$), and that blood was kept at 278.15 K and sent to a medical center for the measurement of the hematocrit value.

At first, the first needle was introduced into the needle launcher shown in Fig. 2, and the passing time of the needle between two magnetic sensors was measured by a programmable controller. After this operation, the falling needle stopped at the bottom of the fluid vessel and was rapidly moved into the space in the needle collector by the guidance of a small magnet from outside the vessel through manual operation. Next, the second needle was also introduced into the needle launcher as soon as possible. When the final needle (eighth needles) was introduced into the needle launcher and the passing time was measured, the rheometric operation was finished. Densities of fresh human blood were measured by a portable density/gravity meter manufactured by Kyoto Electric Co., Ltd., within an uncertainty of $10^{-3}$ g·m$^{-3}$ as soon as possible. Human blood densities for females and males are listed in Table 3.

Table 3 Apparent viscosity, hematocrit value, and density of human blood and rabbit blood at 310.15 K

<table>
<thead>
<tr>
<th></th>
<th>Ht (g·m$^{-3}$)</th>
<th>Density $\times 10^{-3}$ (kg·m$^{-3}$)</th>
<th>Fresh blood anticoagulant (mPa·s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42.8</td>
<td>1.0531</td>
<td>5.96</td>
</tr>
<tr>
<td>Female</td>
<td>38.1</td>
<td>1.0511</td>
<td>4.96</td>
</tr>
<tr>
<td>Rabbit I</td>
<td>40.5</td>
<td>1.0402</td>
<td>3.65</td>
</tr>
<tr>
<td>Rabbit II</td>
<td>45.0</td>
<td>1.0541</td>
<td>4.84</td>
</tr>
</tbody>
</table>

The experimental treatment of the fresh human blood with anticoagulant was carried out within 120 s except for the time needed for taking the blood (about 60 s), that is, the total time needed for this operation for each person was finished within 3 min.

The observed flow curve for male blood with anticoagulant was shown in Fig. 6. This flow curve showed a linear relationship between the shear stress and shear rate in a high shear stress range (150 s$^{-1}$ to 400 s$^{-1}$). However, non-Newtonian behavior (Casson behavior) was confirmed in a low shear stress range (0 to 150 s$^{-1}$). The observed flow curve of fresh blood showed the three typical fluid regions, that is, the non-Newtonian fluid region for the low shear rate range, and the transition region and Newtonian fluid region for the high shear rate range. Figure 7 shows the flow curve of female human blood with anticoagulant and a similar tendency with Fig. 6 as non-Newtonian fluid was obtained. This flow curve also shows a linear relationship between the shear stress and shear rate in the high shear stress range (130 s$^{-1}$ to 400 s$^{-1}$). However, non-Newtonian behavior (Casson behavior) was confirmed in the low shear stress range (0 to 130 s$^{-1}$).
Figure 8 is showing the flow curve of rabbit blood. Similar tendency for flow curve with human blood was obtained. Figure 9 gives the behavior of non-Newtonian regain of low shear ranges in the flow curve for rabbit blood without anticoagulant. This data was obtained from the experiments using special needles controlled similar density with rabbit blood density. It is found that this rheometer can measure flow curve for blood without anticoagulant.

Figure 10 shows the relationship between the apparent viscosity and shear rate for fresh human blood and rabbit bloods at 310.15 K. The apparent viscosity with anticoagulant, the blood viscosity of male blood was higher than that of female blood.

Figure 11 shows the relationship between average viscosity and hematocrit values of fresh human blood at 310.15 K. Male blood has a higher viscosity compared to female blood. A linear relationship with the hematocrit values was obtained. However, the viscosity of apparent viscosity shows down with increasing of hematcrit value. It was found that the viscosity for blood was closely connected with the hematocrit values.
6 Conclusion

A compact-sized falling needle rheometer with quick operation and automatic flow analysis has been developed for the viscometry of human blood and rabbit blood with anticoagulant. The volume of a fresh blood sample only needs to be 4 cm$^3$ and the measuring time is within 3 min after taking a blood sample from the vein. The measured flow properties of both bloods are evaluated as a flow curve, that is, the relationship between the shear stress ($\tau$) and shear rate ($\gamma$). Observed flow curves of fresh human blood show the three typical fluid regions, that is, the non-Newtonian fluid region for the low shear rate range, and the transition region and Newtonian fluid region for the high shear rate range. Flow properties of blood such as the apparent viscosity ($\mu$) in the Newtonian fluid region are measured, and they are compared between male and female blood. It is found that the human blood viscosity of males (6.0 mPa·s to 6.4 mPa·s) shows a higher value than that of females (4.8 mPa·s to 5.3 mPa·s). A linear relationship between the hematocrit value, which is the volume percentage of red corpuscles in the human blood, and the apparent viscosity is observed for male and female blood. Rabbit blood viscosities with anticoagulant were also measured using this falling needle rheometer. It is found that the compact-sized falling needle rheometer presented in this work is very useful for rheometry studies of fresh human blood without anticoagulant.

Nomenclature

- $d$: needle diameter, m
- $f_r$: yield stress of a Casson fluid, Pa
- $g$: gravitational acceleration, m·s$^{-2}$
- $G$: geometric needle constant, 1·m$^{-2}$
- $K$: fluid consistency, Pa·s$^{-n}$
- $k$: ratio of container to needle diameter
- $kR$: needle radius, m
- $L$: total needle length, m
- $n$: fluid index
- $P_1, P_2$: pressure of the upper and lower end of a minute circular cylinder, Pa
- $\Delta P$: pressure difference ($\Delta P = P_1 - P_2$), Pa
- $Q$: net flow rate of fluid pushed aside by the needle, m$^3$·s$^{-1}$
- $r$: radius coordinate, m
- $R$: container radius, m
- $u$: velocity in the system length direction, m·s$^{-1}$
- $U_t$: terminal velocity of a falling needle, m·s$^{-1}$
- $\gamma$: shear rate, s$^{-1}$
- $\mu$: Newton viscosity, Pa·s
- $\pi$: circular constant
- $\rho_f$: fluid density, kg·m$^{-3}$
- $\rho_s$: needle density, kg·m$^{-3}$
- $\tau$: shear stress, Pa
- $\tau_y$: yield stress, Pa

References