Analysis of influence of fibrinogen concentration on blood dielectric properties by GHz electrical impedance spectroscopy

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Abstract The influence of fibrinogen concentration on blood dielectric properties has been analyzed by GHz electrical impedance spectroscopy (EIS). The complex impedances of native blood and blood with various fibrinogen concentrations \(Z_{\text{blood,exp}}^*\) were measured by a coaxial sensor in the frequency range from 1 MHz to 3 GHz. The complex permittivity of native blood and blood with various fibrinogen concentrations \(\varepsilon_{\text{blood}}^*\) were extracted from the \(Z_{\text{blood,exp}}^*\) by equivalent circuit model based on the transmission line theory. The reactance \(X_{\text{blood,native}}\) and resistance \(R_{S_{\text{blood,native}}}\) of native blood have a peak called characteristic frequency \(f_c\) at around 300 MHz. At the time \(t = 0\) min just after fibrinogen addition, the relative blood permittivity \(\varepsilon_{\text{blood}}\) decreases, conductivities \(\sigma_{\text{blood}}\) increases and \(f_c\) shifts to higher frequency with increase of fibrinogen concentrations \(c_{\text{fib}}\) in plasma. With increment of time, from \(t = 0\) min to \(t = 12\) min, \(\varepsilon_{\text{blood}}\) decreases while \(\sigma_{\text{blood}}\) slightly decreases to time because red blood cell (RBC) aggregation reaction. By comparing the \(\varepsilon_{\text{blood,native}}\) of native blood and blood with various \(c_{\text{fib}}\), the fibrinogen dissolved in plasma rises the blood permittivity. However, fibrinogen is unable to rise the blood permittivity unlimitedly because of RBC aggregation reaction.

Keywords fibrinogen, blood, dielectric property, GHz, electrical impedance spectroscopy

1. Introduction

Fibrinogen which is a major protein in blood component to promote red blood cell (RBC) aggregation is identified as a significant independent risk factor for cardiovascular disease [1]. RBC aggregation leads to blood coagulation and thrombosis formation which is an important cause of cardiovascular disease [2, 3]. It is essential to measure the fibrinogen concentrations for monitoring RBC aggregation and for predicting thrombosis formation during routine treatment of cardiovascular disease. Currently, many methods have been used in fibrinogen concentration measurement. As a conventional method, fibrinogen prothrombin-time (PT) is widely used because of high sensitivity of fibrinogen concentration, while fibrinogen Clauss assay is suitable for low fibrinogen concentration levels [4]. As a high sensitivity of quite low fibrinogen concentration, S. Campuzano et al. designed immunosensors for fibrinogen concentration measurement in human plasma, which spends 90 minutes for a total analysis in diluted plasma samples [5]. However, these methods have drawbacks which are the need of labeling, being invasive and time-consuming.

In order to overcome the drawbacks, the electrical impedance spectroscopy (EIS) method has extensively been used for the fibrinogen concentration measurement of blood due to being label-free, non-invasive and high-speed. Spence showed a good correlation between PT derived method and EIS method in fibrinogen concentration measurement by statistical analysis [6]. Zhao et al. investigated the relationships between the impedance and fibrinogen concentration at three frequencies 100 kHz, 800 kHz and 1.2 MHz [7]. Park et al. developed an erythrocyte membrane-draped electrochemical impedance biosensor for wide fibrinogen concentration range measurement of fibrinogen in the plasma sample in the frequency range from 0.1 kHz to 100 kHz [8].

However, the previous researches in kHz and MHz frequency range only demonstrate two kind dielectric dispersion which are \(\alpha\)-dispersion and \(\beta\)-dispersion. The
α-dispersion, which is usually located at kHz frequency range, is generally caused by electric double layer (EDL) \([9, 10]\). The β-dispersion, which is located in MHz frequency range, is caused by polarization of ions surrounding the interfacial of cell’s plasma membrane \([11, 12]\). Besides α and β-dispersion, the γ-dispersion is usually located in over 100 MHz to GHz frequency range. The γ-dispersion is due to the molecules with an abundance of free-electrons polarize by exposure to the electromagnetic fields \([13]\). E. Levy et al. deeply discussed the state of water in aqueous solutions of nonionic solutes and the differences between the dynamics and structure of solutions of salts \([14, 15]\). In recent years, several researches in blood dielectric properties in the over 100 MHz to GHz frequency range has been done by EIS. As a typical example, M. Wolf et al. investigated the α, β and γ-dispersion of human blood in the frequency range from 1 Hz to 40 GHz under temperature and hematocrit value variation \([16]\). F. Bordi et al. analyzed the dielectric properties of RBC suspension in physiological saline solution in the frequency range from 1000 Hz to 1 GHz \([17]\). However, the previous researches over 100 MHz to GHz frequency range only discussed the blood dielectric properties. The influence of fibrinogen concentration on blood dielectric properties in the over 100 MHz to GHz frequency range is not clarified. Thus, for a much broader frequency range from 1 Hz to 40 GHz, it is valuable to investigate the influence of fibrinogen concentration on the blood dielectric properties in the over 100 MHz to GHz frequency range.

Hence, in this study, in order to investigate the influence of various fibrinogen concentrations on blood dielectric properties in the frequency range from 1 MHz to 3 GHz, the complex blood permittivity with various fibrinogen concentrations are extracted. The complex permittivity of blood with various fibrinogen concentrations at 300 MHz and 2 GHz are shown to discuss the change of dielectric properties as the time elapses. The complex permittivity of native blood and blood with fibrinogen are compared to illustrate that the influence of fibrinogen on the blood dielectric properties. The complex blood permittivity with various fibrinogen concentrations are compared to explain that blood dielectric properties change caused by various fibrinogen concentrations.

2. Experimental Setup, Conditions, and Method

2.1. Experimental setup

Fig. 1 shows the GHz impedance measurement setup. It consists of an impedance analyzer (Model IM7587: Hioki E.E Corporation, Japan), a test head, a coaxial sensor and a personal computer. The impedance analyzer is for measurement of impedance. The test head is for connecting with coaxial sensor. The coaxial sensor is a standard SMA (Subminiature version A) coaxial conversion connector (RPSMAJ-SMAP; To-Comme Corporation, Japan). By SMA plug (SMAP) interface, the coaxial sensor is directly screwed to the test head of the impedance analyzer to prevent cable inductance. The coaxial sensor is employed to measure the blood sample by the reverse-polarity SMA jack (RPSMAJ) interface. The total height of coaxial sensor is \(h = 18.3 \text{ mm}\). The internal height of the RPSMAJ interface is \(h_{\text{sol}} = 2.5 \text{ mm}\). The coaxial sensor has an internal axial diameter is \(d_{\text{ip}} = 0.9 \text{ mm}\) and an external cylinder diameter \(d_{\text{out}} = 4.6 \text{ mm}\). The characteristic impedance of the coaxial sensor is \(Z_{\text{char}} = 50 \text{ Ω}\). The personal computer is used to control the impedance analyzer and process the experimental data.

2.2. Experimental Condition

Fig. 2 shows the preparation procedure of six blood samples which are one native blood samples and five blood samples with various fibrinogen concentrations. Fig. 2 (a) shows the preparation of native blood samples which are six test tubes containing \(v_i = 1.5 \text{ mL}\) blood samples at \(Hct = 35%\). As step 1, about 50 mL of native citrated blood of Hematocrit \(Hct = 46%\) was centrifuged to obtain plasma. As step 2, blood with \(Hct = 42%\) was then adjusted to \(Hct = 35%\) by adding plasma of volume \(v_{\text{plasma}} = 0.36 \text{ mL}\) to porcine blood of volume \(v_{\text{blood}} = 1.14 \text{ mL}\) initially appended into six separate test tubes. Fig. 2 (b) shows the preparation of blood samples with various fibrinogen concentrations. As step 1, the five test tubes containing \(v_i = 1.5 \text{ mL}\) native blood samples were centrifuged. As step 2, the plasma of increasing volumes \(v_{\text{suck}} = 0.1, 0.2, 0.3, 0.4 \text{ and } 0.5 \text{ mL}\) sucked out from test tubes 1 to 5 respectively. As step 3, the fibrinogen (Wako...
Pure Chemical Ind, Japan) with mass $g_{blood} = 0.3$ g was added to 6 mL of afore prepared plasma to obtain the fibrinogen-plasma solution (FPS) with concentration $c_{FPS} = 0.05$ g/mL. Then the fibrinogen-plasma solution of volume $v_{injected}$ = 0.1, 0.2, 0.3, 0.4 and 0.5 mL were added into test tubes 1 to 5 respectively. Each were mixed to restore the hematocrit back to $Hct = 35\%$. The fibrinogen concentrations in the blood samples were $c_{fib} = 0.005, 0.010, 0.0150, 0.020$ and 0.025 g/mL under the condition that the original fibrinogen in blood samples was ignored.

The NaCl solutions (Hayashi Pure Chemical Ind, Japan) whose concentration is $c_{NaCl} = 0.01$ mol/L was also prepared to calibrate the coaxial sensor.

Fig. 2 (c) shows a native blood sample or blood with various fibrinogen concentrations $c_{fib}$, (b) blood with various fibrinogen concentrations $c_{fib}$ = 0.005, 0.010, 0.0150, 0.020 and 0.025 g/mL. (c) Measurement condition.

Fig. 2 (a) shows the equivalent circuit model based on the plane wave theory [18] in coaxial sensor including NaCl solution as a calibration or blood sample. The equivalent circuit model has two transmission lines with three types of components, 'NaCl' or 'blood'. The subscripts of the symbols stands for 'cab' as the calibration solution, 'NaCl' or 'blood'.

The experimental measured complex impedance $Z_{NaCl, exp}$ of samples in coaxial sensor is described as

$$ Z_{NaCl, exp} = Z_{NaCl, 0} + Z_{cab, 0} \tan h(\gamma_{NaCl, 0} h_{cab}) $$

where $h_{cab}$ is the known the transmission line length [m], $Z_{cab, 0} = 50 \Omega$ is the characteristic impedance of the coaxial sensor [Ω]. Complex propagation constant of coaxial sensor $\gamma_{cab}$ is a unique unknown value.

The complex impedance of calibration solution $Z_{NaCl}$ in the case of open end of the transmission line is calculated by

$$ Z_{NaCl} = Z_{NaCl, 0} \tan h(\gamma_{NaCl, 0} h_{sol}) $$

where $h_{sol}$ is the known solution height in the coaxial sensor.
The complex propagation constant $\gamma_{\text{NaCl}}^*$ and characteristic impedance $Z_{\text{NaCl},0}^*$ of NaCl solution are calculated from

$$\gamma_{\text{NaCl}}^* = \sqrt{j \omega \mu_{\text{NaCl}} (\varepsilon_{\text{NaCl}} + j \omega \varepsilon_{\text{NaCl}})}$$  \hspace{1cm} (4)$$

$$Z_{\text{NaCl,0}}^* = \frac{1}{G_f} \sqrt{j \omega \mu_{\text{NaCl}} (\varepsilon_{\text{NaCl}} + j \omega \varepsilon_{\text{NaCl}})}$$ \hspace{1cm} (5)$$

where the permeability of NaCl solution is assumed as $\mu_{\text{NaCl}} = \mu_0 = 4 \times 10^{-7}$ H/m. The relative permittivity $\varepsilon_{\text{NaCl}}$ and conductivity $\sigma_{\text{NaCl}}$ were function of NaCl concentration obtained from reference literature [19, 20]. $j$ is the imaginary unit, and $\omega$ is angular frequency [rad/s]. $G_f = 2\pi \ln(r_i/r_o)$ is the geometric parameter of coaxial sensor with the axial cross-section of internal radius $r_i$ and external radius $r_o$. According to [17], the influence of the electrode polarization is mainly in frequency range lower than 1 MHz. By measuring NaCl solution with different concentrations and comparing with their known complex permittivity, we find that the calibration method is able to reliably eliminate the influence of coaxial sensor.

Fig. 3 (c) shows the procedure of calculation of complex blood permittivity $\varepsilon_{\text{blood}}^*$ from the the complex propagation constant $\gamma_{\text{cab}}^*$ calculated in the calibration procedure.

The experimentally measured complex impedance of blood $Z_{\text{blood,exp}}^*$ is described as

$$Z_{\text{blood,exp}}^* = Z_{\text{cab,0}}^* + Z_{\text{cab,0}}^* \tanh(\gamma_{\text{cab}}^* h_{\text{cab}}) + Z_{\text{blood,0}} + Z_{\text{blood,0}} \tanh(\gamma_{\text{blood}}^* h_{\text{sol}})$$ \hspace{1cm} (6)$$

where the impedance of blood in coaxial sensor $Z_{\text{blood}}^*$ is expressed as

$$Z_{\text{blood}}^* = \frac{Z_{\text{blood,0}}^*}{\tan h(\gamma_{\text{blood}}^* h_{\text{sol}})}$$ \hspace{1cm} (7)$$

where complex propagation constant $\gamma_{\text{blood}}^*$ and characteristic impedance $Z_{\text{blood,0}}^*$ of the blood are defined as the function of $\varepsilon_{\text{blood}}^*$ and $\mu_{\text{blood}}$

$$\gamma_{\text{blood}}^* = j \omega \sqrt{\mu_{\text{blood}} \varepsilon_{\text{blood}}^*}$$ \hspace{1cm} (8)$$

$$Z_{\text{blood,0}}^* = \frac{1}{G_f} \sqrt{\frac{\mu_{\text{blood}}}{\varepsilon_{\text{blood}}^*}}$$ \hspace{1cm} (9)$$

By combining the equations (7), (8), and (9), the complex blood permittivity with fibrinogen $\varepsilon_{\text{blood}}^*$ are calculated under the condition of permeability of blood $\mu_{\text{blood}} = \mu_0 = 4 \times 10^{-7}$ H/m. The relative permittivity $\varepsilon_{\text{blood}}$ and conductivity $\sigma_{\text{blood}}$ were function of blood concentration obtained from reference literature [19, 20]. $j$ is the imaginary unit, and $\omega$ is angular frequency [rad/s]. $G_f = 2\pi \ln(r_i/r_o)$ is the geometric parameter of coaxial sensor with the axial cross-section of internal radius $r_i$ and external radius $r_o$. According to [17], the influence of the electrode polarization is mainly in frequency range lower than 1 MHz. By measuring NaCl solution with different concentrations and comparing with their known complex permittivity, we find that the calibration method is able to reliably eliminate the influence of coaxial sensor.

Fig. 3 (c) shows the procedure of calculation of complex blood permittivity $\varepsilon_{\text{blood}}^*$ from the the complex propagation constant $\gamma_{\text{cab}}^*$ calculated in the calibration procedure.

The experimentally measured complex impedance of blood $Z_{\text{blood,exp}}^*$ is described as

$$Z_{\text{blood,exp}}^* = Z_{\text{cab,0}}^* + Z_{\text{cab,0}}^* \tanh(\gamma_{\text{cab}}^* h_{\text{cab}}) + Z_{\text{blood,0}} + Z_{\text{blood,0}} \tanh(\gamma_{\text{blood}}^* h_{\text{sol}})$$ \hspace{1cm} (6)$$

where the impedance of blood in coaxial sensor $Z_{\text{blood}}^*$ is expressed as

$$Z_{\text{blood}}^* = \frac{Z_{\text{blood,0}}^*}{\tan h(\gamma_{\text{blood}}^* h_{\text{sol}})}$$ \hspace{1cm} (7)$$

where complex propagation constant $\gamma_{\text{blood}}^*$ and characteristic impedance $Z_{\text{blood,0}}^*$ of the blood are defined as the function of $\varepsilon_{\text{blood}}^*$ and $\mu_{\text{blood}}$

$$\gamma_{\text{blood}}^* = j \omega \sqrt{\mu_{\text{blood}} \varepsilon_{\text{blood}}^*}$$ \hspace{1cm} (8)$$

$$Z_{\text{blood,0}}^* = \frac{1}{G_f} \sqrt{\frac{\mu_{\text{blood}}}{\varepsilon_{\text{blood}}^*}}$$ \hspace{1cm} (9)$$

By combining the equations (7), (8), and (9), the complex blood permittivity with fibrinogen $\varepsilon_{\text{blood}}^*$ are calculated under the condition of permeability of blood $\mu_{\text{blood}} = \mu_0 = 4 \times 10^{-7}$ H/m.
\( \times 10^{-7} \text{ H/m}. \) The complex permittivity of the blood is expressed as permissivity \( \varepsilon_{\text{blood}} \) and conductivity \( \sigma_{\text{blood}} \) as

\[
\varepsilon^*_{\text{blood}} = \varepsilon_{\text{blood}} - j\sigma_{\text{blood}} / \omega \varepsilon_0
\]

where \( \varepsilon_0 \) is the vacuum permittivity [H/m].

3. Experimental Results

3.1. The impedance of native blood and blood with various fibrinogen concentrations at time \( t = 0 \text{ min} \)

Figs. 4 (a) (b) and (c) show the impedance results \( Z^*_{\text{blood}} \) of native blood and blood with five fibrinogen concentrations \( c_{\text{fib}} \) at time \( t = 0 \text{ min} \). According to Fig. 4 (a), the reactance \( X_{\text{blood}} \) decreases with the increase of fibrinogen concentration \( c_{\text{fib}} \) in the blood. It is evident that \( X_{\text{blood}} \) is smaller than blood containing more dissolved fibrinogen \( X_{\text{blood},c} \). The relationship between \( X_{\text{blood}} \) and \( c_{\text{fib}} \) also obeys this regularity even in GHz frequency range. However, in the frequency range \( f \geq 1 \text{ GHz} \), reactance values \( X_{\text{blood},c} \) are too close to each other and there is a barely noticeable difference among the various \( c_{\text{fib}} \). Fig. 4 (c) shows the resistance of blood \( R_{s_{\text{blood}}} \) decreases with the increase of fibrinogen concentration \( c_{\text{fib}} \) and frequency \( f \).

The blue points of Figs. 4 (a) and (b) represent the minimum value of \( X_{\text{blood}} \). The frequency point at which the reactance exhibits the minimum values of \( X_{\text{blood}} \) are considered as the characteristic frequency \( f_c \). The \( f_c \) of native blood and blood with five fibrinogen concentrations increases with the increase of \( f = 300 \text{ MHz} \) to 400 MHz with the increase of \( c_{\text{fib}} \). Under the condition that frequency \( f \) larger than \( f_c \), \( X_{\text{blood},c} \) increase with the increase of \( c_{\text{fib}} \). Besides, in Fig. 4 (b), there is nearly no contact impedance caused by EDL, especially in the frequency range larger than \( f = 10 \text{ MHz} \) as discussed in reference literature [21].

Figs. 5 (a) and (b) show the reactance \( X_{\text{blood}} \) and resistance \( R_{s_{\text{blood}}} \) of blood with various fibrinogen concentrations at \( f = 300 \text{ MHz} \) and \( t = 0 \text{ min} \). The \( X_{\text{blood}} \) increases and \( R_{s_{\text{blood}}} \) decreases as the concentration of fibrinogen \( c_{\text{fib}} \) increases according to Figs. 5 (a) and (b). The \( c_{\text{fib}} \) has a clear linear relationship with both \( X_{\text{blood}} \) and \( R_{s_{\text{blood}}} \). Figs. 5 (a) and (b) does not include resistance \( R_{s_{\text{blood,native}}} \) and reactance \( X_{\text{blood,native}} \) of native blood because the native blood also contains fibrinogen. While, the native blood’s fibrinogen concentration is not different from the \( c_{\text{fib}} \) that we added.
3.2. Dielectric property of blood with increasing fibrinogen

Figs. 6 (a) and (b) show the relative permittivities \( \varepsilon_{\text{blood/native}} \) and conductivity \( \sigma_{\text{blood/native}} \) of native blood and blood with increasing fibrinogen concentrations which are calculated based on equations (7), (8), and (9). Compared with \( Z_{\text{blood}}^* \), relative permittivities \( \varepsilon_{\text{blood}} \) are unable to show a clear difference among the blood with various \( c_{\text{fib}} \). Fig. 6 (a) shows the relative blood permittivity \( \varepsilon_{\text{blood}} \) reduce with an increase of frequency \( f \). The relative permittivity \( \varepsilon_{\text{blood}} \) first decreases sharply from \( f = 1.0 \text{ MHz} \) up to around \( f = 100 \text{ MHz} \) while before decreasing gradually up to \( f = 3 \text{ GHz} \).

From Fig. 6 (b), it is clear that the conductivity \( \sigma_{\text{blood}} \) of blood with higher \( c_{\text{fib}} \) is larger than blood with lower \( c_{\text{fib}} \) in all frequency range. The \( \sigma_{\text{blood}} \) sharply increases from around \( f = 1 \text{ GHz} \) to \( f = 3 \text{ GHz} \) which is attribute to the relaxation of water in the GHz frequency range [14, 15].

As shown in Fig. 7 (a), the \( \varepsilon_{\text{blood}} \) decreases as the fibrinogen concentrations \( c_{\text{fib}} \) increasing. Fig. 7 (b) illustrates that the blood conductivity \( \sigma_{\text{blood}} \) increases as \( c_{\text{fib}} \) increases. This means that blood with higher \( c_{\text{fib}} \) includes much more ions than blood with lower \( c_{\text{fib}} \).

4. Discussions

4.1. Change of blood permittivity and conductivity with time

According to Fig. 4 (a), the curves of Nyquist plot between blood with various fibrinogen concentrations \( c_{\text{fib}} \) show the most apparent difference that exists around \( f = 300 \text{ MHz} \) which is also located in the \( \beta \)-dispersion frequency range. Besides, the \( \gamma \)-dispersion, which is results of molecules’ reorientation in plasma, usually exists \( f > 1 \text{ GHz} \). Fig. 8 illustrates the changes in relative permittivity \( \varepsilon_{\text{blood/native}} \) and conductivity \( \sigma_{\text{blood/native}} \) of native blood and blood with \( c_{\text{fib}} = 0.005 \text{ g/mL} \) and \( c_{\text{fib}} = 0.025 \text{ g/mL} \) as the increasing of time at \( f = 300 \text{ MHz} \) and \( f = 2 \text{ GHz} \). In Fig. 8 (a), compared with the \( \varepsilon_{\text{blood/native}} \), the permittivities of the blood with fibrinogen \( \varepsilon_{\text{blood,c}=0.005} \) and \( \varepsilon_{\text{blood,c}=0.025} \) also show linearly decreasing tendency in the whole measured time-range. However, \( \varepsilon_{\text{blood,c}=0.005} \) and \( \varepsilon_{\text{blood,c}=0.025} \) are higher than \( \varepsilon_{\text{blood/native}} \) since time \( t = 0 \text{ min} \). The blood permittivity \( \varepsilon_{\text{blood}} \) shows a linearly decreasing tendency with an increment of time at both \( f = 300 \text{ MHz} \) and \( f = 2 \text{ GHz} \) as shown in Figs. 8 (a) and (c). Baskurt and Meiselman point out that RBC are able to aggregate in aqueous solutions containing fibrinogen at stasis.
Meanwhile, the RBC aggregation is a relatively fast process. Red blood cells aggregate to form two-dimensional structures which is termed rouleau or rouleaux. Rouleaux can form three-dimensional structures in larger geometries. It is about seconds for initial rouleau formation while it is somewhat longer for forming three-dimensional aggregates. Thus, Figs. 8 (a) and (c) show that $\varepsilon_{\text{blood}}$ linearly decreases with the aggregation of RBC.

Fig. 8 (b) shows that the blood conductivity $\sigma_{\text{blood}}$ slightly decreases as the time increasing. The blood conductivity with additional fibrinogen $\sigma_{\text{blood},c}=0.005$ and $\sigma_{\text{blood},c}=0.025$ are larger than the blood conductivity $\sigma_{\text{blood}}$. Meanwhile, $\sigma_{\text{blood},c}=0.005$ and $\sigma_{\text{blood},c}=0.025$ slightly and linearly decrease over time at $f=300$ MHz and $f=2$ GHz. This shows that fibrinogen increases the blood conductivity. A pH titration on fibrinogen shows that it has a net charge of $-7$ at a pH $= 7$ and therefore it is a protein that is highly negatively charged and soluble in water [22]. An increase in fibrinogen concentration provides more ionizable negatively charged groups leading to an increased electrical conductivity. The $\sigma_{\text{blood}}$ decreases over time illustrate that the surface ions participate in the reaction when blood cells aggregate.

### 4.2. Reason for permittivity and conductivity change

Based on the experimental results, the influence of fibrinogen on the blood dielectric properties is able to be explained in two aspects, which are the dielectric properties of fibrinogen in plasma and RBC aggregation caused by fibrinogen.

One of the reasons is the dielectric properties of fibrinogen in the plasma. The plasma as a vital component of blood is made up of the water, proteins, carbohydrates and electrolytes. Proteins include albumin, globulins, fibrinogen, mucoprotein, and so on. On one side, the free and bound water in plasma are crucial to the study of dielectric properties, which has been discussed in [16, 23]. On the other side, our previous research has illustrated that fibrinogen plays a vital role in dielectric properties of blood rather than albumin [24], although more than 60% of total protein concentration in plasma is albumin [23]. As shown in Fig. 8 (a), the blood permittivity $\varepsilon_{\text{blood}}$ increases significantly after part of plasma is replaced by fibrinogen plasma solution under the same hematocrit $Hct=35\%$. It shows that the fibrinogen as a typical protein has a relatively high permittivity compared with the plasma. Thus, the plasma with fibrinogen increases the permittivity of native blood. The researches on other proteins also support that organic
molecules such as amino acids have high permittivity [25]. The loss of these molecules reduces cytoplasmic permittivity. Pitera, J et al. shows that permittivity is different under different conditions of pH, temperature, solvation, or ligand binding [26]. H. Li et al. points out that the cytosolic dielectric constant changes with its composition and increases significantly with protein concentration based on the simulation results [27]. However, according to the Fig. 8, the blood permittivity $\varepsilon_{\text{blood}}$ is unable to be increased indefinitely by only increasing the fibrinogen concentration $c_{\text{fib}}$. As shown in Figs. 8 (a) and (c), the $\varepsilon_{\text{blood}}$ of $c_{\text{fib}} = 0.005$ g/mL increases as compared with $\varepsilon_{\text{blood, native}}$ of native blood. However, the blood with fibrinogen concentrations $c_{\text{fib}} = 0.025$ g/mL has a lower permittivity than the blood with $c_{\text{fib}} = 0.005$ mL g/mL. This is shows adding much more fibrinogen does not mean the $\varepsilon_{\text{blood}}$ is unlimitedly increasing.

The other reason is the RBC aggregation caused by fibrinogen. Baskurt and Meiselman point out that fibrinogen is the major plasma component promoting RBC aggregation in blood, with an almost linear relationship between aggregate size and plasma fibrinogen concentration [3]. As shown in Fig. 7 (a), the blood with a higher $c_{\text{fib}}$ has a smaller permittivity. Meanwhile, Fig. 8 (a) shows the permittivity of the blood $\varepsilon_{\text{blood}}$ decreases as the time increasing. The reduction of permittivity involves the process of rouleaux formation and RBC aggregation. Although the mechanisms of RBC aggregation have not been fully explained [3], the aggregation process is significantly affected by surface properties and structure of RBC [7, 28, 3]. The two factors lead to rouleaux formation. Due to the rouleaux formation or aggregation, the distribution of RBC in the plasma is changed. The change distribution of RBC also leads to the change of the electrical field in the coaxial sensor and measured impedance. By comparing the Fig. 7 (a) and Fig. 8 (a), we also find that the change of blood permittivity $\varepsilon_{\text{blood}}$ caused by fibrinogen is much visible than that caused by the RBC aggregation.

Besides, as shown in Fig. 6 (b), the blood conductivity $\sigma_{\text{blood}}$ increase obviously after the plasma in the blood is replaced by fibrinogen plasma solutions. However, $\sigma_{\text{blood}}$ of blood shows much apparent change with increment of $c_{\text{fib}}$ than the change caused by increment of time as shown in Fig. 7 (b) and Fig. 8 (b). It illustrates that $c_{\text{fib}}$ is the main reason for the change of $\sigma_{\text{blood}}$. Blood resistance $R_{\text{blood}}$ gradually decreases with the increment of $c_{\text{fib}}$ according to Fig. 5 (b).

5. Conclusions

In this study, the impedance of native blood and blood with various fibrinogen concentrations have been measured in the frequency range from 1 MHz to 3 GHz. The permittivity and conductivity of blood as they vary with the fibrinogen concentration are extracted from the measured impedance. From the discussion, the following specific conclusions are obtained:

1. The permittivity of blood with various fibrinogen concentrations $\varepsilon_{\text{blood}}$ linearly decreases with the increment of time. Meanwhile the blood conductivity $\sigma_{\text{blood}}$ slightly decreases. This is because the RBC aggregate at stasis and the surface ions participate in the aggregation reaction.

2. The permittivity $\varepsilon_{\text{blood}}$ and conductivity $\sigma_{\text{blood}}$ of blood with fibrinogen are higher than the native blood permittivity and conductivity due to permittivity of fibrinogen in plasma. $\varepsilon_{\text{blood}}$ linearly decrease with the increase of added fibrinogen concentration $c_{\text{fib}}$ due to the RBC aggregation.

3. The plasma with fibrinogen increases the permittivity of native blood. However, fibrinogen is unable to rise the plasma permittivity unlimitedly. Injecting much more fibrinogen plasma solution reduces the blood permittivity $\varepsilon_{\text{blood}}$ which illustrates rouleaux formation and RBC aggregation reaction.

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