Monitoring changes of viscoelasticity during blood coagulation with acoustic sensors

Glen Guhr, Raimund Brünig, Hagen Schmidt

Leibniz Institute for Solid State and Materials Research Dresden, Dresden, Germany

Siegmund Gehrisch, Gabriele Siegert

Institute for Clinical Chemistry and Laboratory Medicine, TU Dresden
Dresden, Germany

Manfred Weihnacht

InnoXacs, Dippoldiswalde, Germany

***Abstract*—In our previous work we demonstrated the general suitability of surface and bulk acoustic wave (SAW and BAW) structures for recording the continuous change of fluidic behavior in the course of blood coagulation processes [1]. Special advantage can be taken from the direct impact of fluid consistence on SAW and BAW device parameters (e.g. resonator frequency, quality factor, impedance). Beside the ability of continuous detection of coagulation process further tests can be realized with SAW and BAW devices as was shown in an acoustic version of thrombelastography [2].**

In this paper, we make the proposal to describe the observed features of blood in the frame of non-Newtonian behavior. Therefore we distinguish between acoustic monitoring of shear viscosity and shear elasticity changes during the whole process of haemostasis. The goal is to get more detailed insight into the ongoing biological process. That approach is suggested from modeling of resonance-antiresonance behavior of thickness shear-mode resonators (TSM), also called quartz crystal microbalance (QCM). The response of resonance and antiresonance parameters can be significantly different from each other, depending on the shear viscosity and elasticity of the loaded fluid. For example, the frequency shifts of both resonances have the same or the opposite sign when changing the viscosity or the elasticity, respectively. The results of modeling the QCM response to viscoelastic loading are used for the interpretation of time dependent measurement signals of the coagulation process.

I. Introduction

In medical diagnostics, among others, blood is one of the most widely investigated human fluids. Much research is focussed on the blood coagulation system. As result a large variety of blood coagulation tests for example the so called Quick-test, the activated partial thromboplastin time (aPTT) or recalcification time have been created. All these tests measure the time that it takes for a certain path of the coagulation cascade until the final step of coagulation, fibrin formation, occurs. Today, all these tests mostly are realized as endpoint assays measuring the time until a coagulating blood sample stops the motion of a rotating steel ball placed inside the sample. But with these tests it is impossible to get information about the viscoelastic changes during coagulation process.

In the last years our group and others reported a novel technique, the quartz crystal microbalance (QCM) technique of being valid for continuous monitoring of the coagulation process [1] [3] [4] (Fig. 1). At such devices mainly the fibrin formation initiates a characteristic change of the resonant frequency vs. time. The total change of resonant frequency gives a clue for the strength and stability of the clot. But until now, no direct value for the change of the viscosity is given.

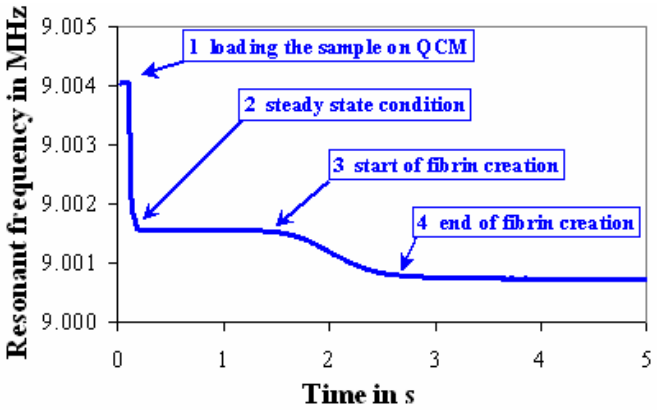

Figure 1. Resonant frequency vs. time as it changes during an activated thromboplastin time coagulation test.

The use of various measuring methods with either BAW devices or SAW devices allows the extraction of certain fluid parameters from the experimental data, independently from the wave type. In this paper, we start with that intention by analyzing the QCM method. Physical models for the use of QCM for liquids are known for a long time [5]. Kanazawa's formula relates the resonant frequency shift to the viscosity of a fluidic load. However, blood is known as a fluid which does not follow the Newton model. In the following we re-examine the physical model of QCM under the assumption that the adjacent fluid is viscoelastic, i.e., we have to introduce shear elasticity in addition to viscosity.

II. Simulation of QCM in Contact with Viscoelastic Fluid

In one-dimensional approach the electrical admittance Y of a liquid loaded QCM plate of thickness d, static capacitance C, shear wave velocity v, piezoelectric coupling coefficient K^2 as a function of reduced frequency $x = \pi d f / v$ (f = frequency) is given by formula (1). A periodic time dependence $\sim\exp(-i\omega t)$ is assumed ($\omega = 2\pi f$).

$$Y = i\frac{2vC}{d}\frac{x}{1-K^2\dfrac{\tan(x)/x}{1-\dfrac{F_{load}\tan(x)}{1+F_{load}/\tan(x)}}} \tag{1}$$

The piezoelectric coupling coefficient K^2 is given by $K^2 = e_{35}^2/(c_{55}^D\varepsilon_3\varepsilon_0)$, with ε_{35} = piezoelectric constant, c_{55}^D = elastic shear modulus at constant dielectric displacement D, ε_3 = relative and ε_0 = absolute dielectric constant. F_{load} is a parameter representing the liquid loading of QCM and is determined by Z_F and Z_Q as the acoustic impedances of fluid and QCM, respectively, according to (2).

$$F_{load} = i\frac{Z_F}{2Z_Q} \tag{2}$$

The acoustic impedances Z_F and Z_Q of both media the fluid and the quartz crystal are given by

$$Z_F = \sqrt{\rho_F G_F} \tag{3a}$$

and

$$Z_Q = \sqrt{\rho_Q c_{55}^D}\,, \tag{3b}$$

respectively, with the mass densities ρ_F and ρ_Q and the complex shear modulus G_F of liquid.

The magnitude of electrical impedance Y^{-1} according to formula (1) as a function of frequency f exhibits the well known maximum-minimum (resonance-antiresonance) behavior depicted in Fig. 2. The curves of Fig. 2 correspond to 3 different viscosity values of the liquid being in contact with the QCM. For a pure viscous fluid we have a shear modulus $G_F = -2\pi i f\eta$ whereas η is the viscosity. The down-shifting of resonant frequency with viscosity has found many applications in the use of QCM for fluid characterization.

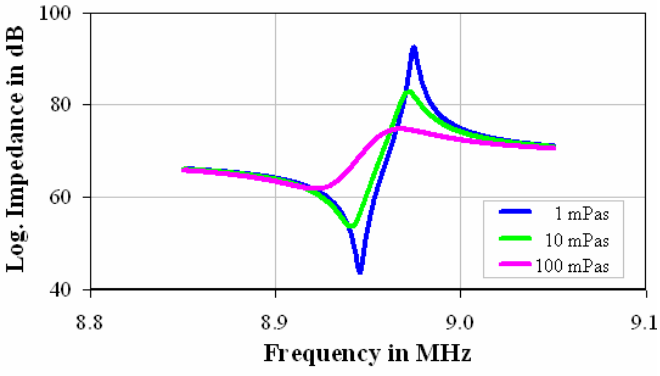

Figure 2. Magnitude of electrical impedance $1/|Y|$ of a liquid loaded QCM as a function of frequency for 3 different viscosities of 1, 10, and 100 mPas. The impedance minimum corresponds to the resonance, the maximum to the antiresonance.

Let us now move to fluids which are not purely viscous. For example, the well established Maxwell model replaces the viscosity η with the complex-valued frequency dependent expression

$$\eta = \frac{\eta_0}{1-i\omega\tau}\,. \tag{4}$$

Introducing a zero frequency viscosity η_0 and a relaxation time τ, we will only write

$$G_F = c_F - i\omega\eta\,, \tag{5}$$

adding an elastic real part c_F to the viscous imaginary part of the shear modulus G_F of liquid. Thus, the Maxwell model (4) is contained as a special frequency dependent case.

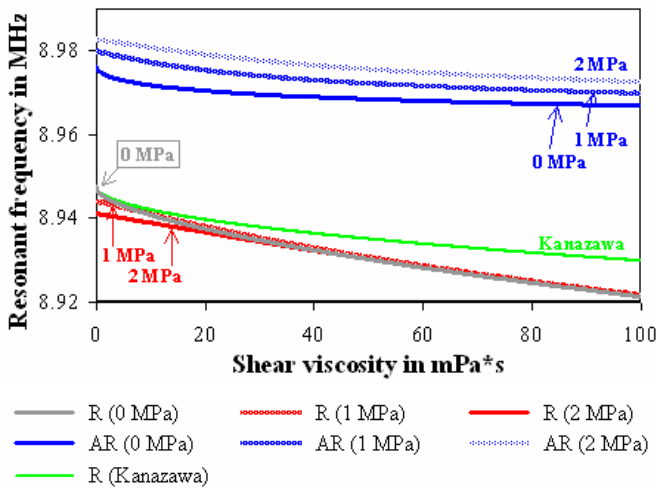

Figure 3. Resonant (R) and antiresonant (AR) frequencies as functions of viscosities for the 3 different shear elasticities of 0, 1 and 2 MPa. The green line corresponds to resonant frequencies according to the Kanazawa formula [5].

The shifting of resonant frequency with viscosity under the additional influence of shear elasticity c_F is distinctly altered (Fig. 3) compared to pure viscous liquids. Besides, the antiresonant frequency increases with elasticity for small viscosities by contrast to the resonant frequency. The indicated value of 1 MPa for the curve parameter "elasticity" in Fig. 3 corresponds to a viscosity of 18 mPas at a QCM frequency of 9 MHz. The curve representing the widely used approximation of Kanazawa [5] is also shown (green chart in Fig. 3).

The numerical results of Fig. 3 suggest to make use of both the shifting of resonant and antiresonant frequencies in order to evaluate a viscoelastic liquid when bringing it in contact with a QCM. That will be attempted in the following for loading a QCM with blood and for the subsequent coagulation process.

III. Blood Coagulation Experiments and Discussion

Experiments have been carried out with commercial available AT-cut quartz thickness shear-mode resonators from MaxTek Inc. The fundamental mode of these devices had a resonant frequency of 9 MHz. These QCM have a total diameter of 25 mm. The diameter of the rear electrode is 6.35 mm and of the working electrode is 12.7 mm. The gold electrodes have a thickness of 120 nm with 20 nm chromium as connecting layer.

Experiments have been started with the measurement of the QCM impedance to get a more detailed overview of its resonance behavior. The blue chart in Fig. 4 shows the log. impedance of a 9 MHz MaxTek Inc. QCM under unloaded conditions. Seven resonance and antiresonance pairs can be observed at a small frequency band slightly above 9 MHz. One can assume, that all these resonance modes are of shear type. For easier discussion we have marked the resonances with numbers from Resonance 1 (R1) to Resonance 7 (R7) and the related antiresonances from antiresonance 1 (AR1) to antiresonance 7 (AR7). R1 has the lowest impedance. This resonance can be considered as the main thickness shear-mode of the QCM. It is well suited for doing coagulation tests and other experiments. The further pairs of resonance and antiresonance are spurious oscillation modes. They appear if there are any abnormalities of the symmetric structure of the quartz crystal and the vapor-deposited metal electrodes. Such abnormalities can be created by differences in the crystal thickness, irregularities of the electrode distance and by the electrode current supply, and divergencies of the circularity of the crystal and the electrodes, for example. According to the relatively small frequency range where all modes have been found, they all should be thickness shear-modes.

The green chart in Fig. 4 shows the impedance of a blood loaded QCM. Loading the sensor with a fluid causes a strong damping of all resonances. The main resonance (R1) and the fourth resonance (R4) are clearly detectable under loading conditions. All of the other resonances are almost completely damped away.

Self-written programs in HP-VEE had been used to monitor the behavior of the resonances and antiresonances vs. time. Therefore the minimum and maximum search of the network analyzer was used. At the points found in this way, 0.5 dB bandwidth detection was carried out and the middle frequency of this bandwidth was recorded. Especially under loaded conditions we had to face problems with the clear detection of AR1. In the unloaded condition, R2 is 500 Hz above AR1. With the loaded QCM it was not possible clearly to detect AR1, it was just to small and overlaid by R2 and AR2. For that reason the measurement program always tracked and recorded the slightly higher positioned AR2. Therefore we decided to record the behavior of thickness shear-mode R4 and AR4 during blood coagulation process. This pair of resonance and antiresonance is better isolated from the other modes. This mode actually does not exactly fit to our model. Nevertheless it is used to demonstrate the way of extracting viscosity and elasticity values from the investigated fluid.

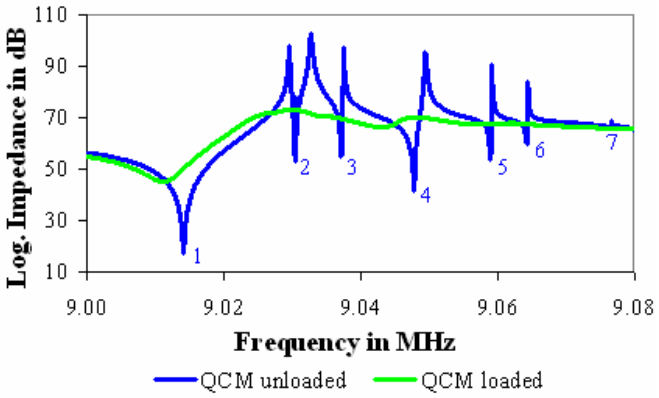

Figure 4. Log. impedance vs. resonant frequency. The blue line shows the measured data of unloaded QCM and the green curve of the blood loaded. The numbers at the impedance minima are indicating the number of the resonances as introduced in the text. Loading the QCM with a fluid causes a significant damping.

Experiments have been done with fresh whole blood samples obtained from a donor. Samples were not older than 6 h. The viscosity of blood depends on the properties and amount of erythrocytes, leukocytes and thrombocytes and the viscosity of the blood plasma. Containing cells and a large number of enzymes, blood is assumed as non-Newtonian fluid. In Fig. 5 the resonant frequency shift of a blood coagulation test, recorded with a 9 MHz QCM is shown. The test performed was the so called activated partial thromboplastin time test and it was carried out at room temperature. Therefore 200 µl whole blood and 100 µl of starting reagents where mixed together to initiate the coagulation process. At the time point, when reagents and blood sample were brought together, the measurement was started while the QCM is still unloaded. The mixing procedure took 6 to 8 s. Afterwards 50 µl of this mixture were dropped on the sensor. Directly afterwards a cover made of polydimethylsiloxane (PDMS) was mounted to prevent the sample from drying and evaporation effects [2].

The blue chart in Fig. 5 shows the behavior of resonance 4 and the red chart of antiresonance 4 as it was measured during

a blood coagulation process. The loading causes a big resonant frequency shift of R4 (about 4.5 kHz) and a smaller frequency shift of AR4 (almost 1 kHz). Afterwards neither R4 nor AR4 are changing during the steady state condition. In this time, the coagulation cascade is in progress but no viscosity change is taking place. First, when it comes to the creation of fibrin, a further decrease of both resonances can be seen. That process starts approximately after 1 min and it comes to its end after 1.7 min. During fibrin formation the viscosity of the sample changes from a fluidic to a more or less gel like shape. This viscosity change causes the decrease of resonant frequency. No more frequency changes take place when the fibrin creation is finished.

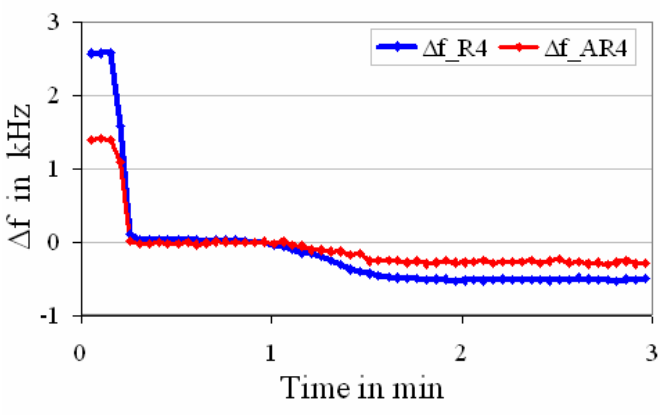

Figure 5. Change of resonant and antiresonant frequency vs. time, measured with a 9 MHz QCM at the 4th resonant and antiresonant frequency. As initial value for the resonant frequency change, the whole blood samples steady state condition observed after the loading was taken.

Finally we want to apply the measured resonant and antiresonant frequency shifts to determine the values of elasticity and viscosity by the use of the above presented model. In Fig. 6 is shown how shear elasticity and viscosity values can be extracted from resonant and antiresonant frequency shifts. Taking the average values of 3 measurement points at each characteristic frequency change of the chart in Fig. 5, we found loading caused frequency descents of 2547 Hz and 1420 Hz for the resonance and antiresonance, respectively. The coagulating blood sample initiated a further decreasing of 545 and 245 Hz. The green circles in Fig. 6 mark the crossings of corresponding curves of constant frequency shift counted from unloaded state. At these points the values for shear elasticity and shear viscosity can be determined. When ignoring elasticity the viscosities before (low value) and after coagulation (higher value) indicated by the squares would be determined. The results of Kanazawa's approximation are also shown (green triangles).

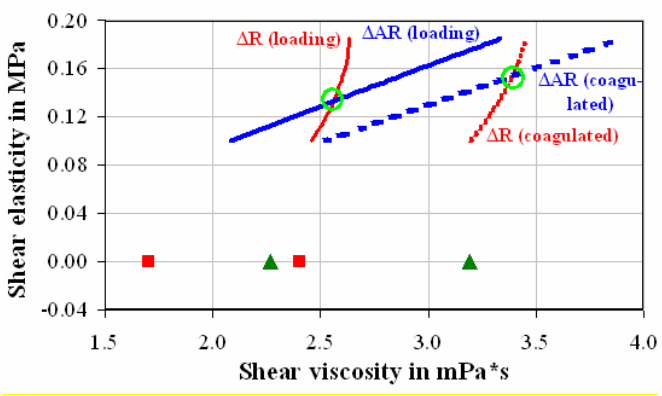

Figure 6. Determination of shear elasticity and viscosity of blood from QCM resonant and antiresonant frequency shifts. Red and blue charts have been calculated with the measured the measured resonant and antiresonant frequency shifts. The crossing points (marked with green circles) of resonance and dotted antiresonance curves mark the values for elasticity and viscosity before and after coagulation process. Viscosities derived from resonant frequencies assuming pure viscosity (zero elasticity) are marked by red squares. The Kanazawa approximation is marked with green triangles.

IV. Conclusions

The model of electrical admittance for a liquid loaded QCM has been exploited for a numerical analysis of the dependencies of resonant and antiresonant frequencies on viscoelastic fluid properties. On that basis, measured data of resonant and antiresonant frequency shifts were used to extract shear elasticities and viscosities separately. Measurements have been realized with blood samples, monitoring the whole coagulation process from loading the QCM until end of coagulation. The model also can be extended for the use at measurement results obtained with SAW devices.

V. Acknowledgement

The authors would like to thank the BMBF that supported this work under the grant 03IP610.

References

[1] G. Guhr, R. Kunze, G. Martin, H. Schmidt, M. Weihnacht, S. Gehrisch, and G. Siegert, "Monitoring blood coagulation with QCM and SH-SAW sensors", 2005 IEEE Ultrasonics Symposium Proc., pp. 58-61.

[2] G. Guhr, R. Kunze, G. Martin, H. Schmidt, M. Weihnacht, S. Gehrisch, and G. Siegert, "Thrombelastography using acoustic sensors", 2006 IEEE Ultrasonics Symposium Proc., pp. 544-547.

[3] H. Muramatsu, K. Kimura, T. Ataka, R. Homma, Y. Miura, I. Karube: A quartz crystal viscosity sensor for monitoring coagulation reaction and its application to a multichannel coagulation detector. Biosensors & Bioelectronics 6 (1991) 353-358.

[4] H.L. Bandey, R.W. Cernosek, W.E. Lee III, L.E. Ondrovic: Blood rheological characterization using the thickness-shear mode resonator. Biosensors and Bioelectronics 19 (2004) 1657-1665.

[5] K.K. Kanazawa, J.G. Gordon: The oscillation frequency of a quartz resonator in Kontakt with a liquid. Analytica Chimica Acta, 175 (1985) 99-105.