

Applicability of a Sensitivity-enhanced Quartz Crystal Microbalance in Analyzing Blood Plasma Viscosity and Coagulation

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(Received December 8, 2021; accepted March 9, 2022)

Keywords: QCM, sensitivity enhancement, blood coagulation, coagulation time, plasma viscosity, portable coagulometer

In this paper, a fundamental frequency of 13 MHz and a thickness-increased n-m-type electrode are adopted to improve the frequency response of a quartz crystal microbalance (QCM) to blood coagulation. The thrombin time (TT) and viscosity of standard plasma were measured with this specially designed QCM at different reaction temperatures, and the results were compared with those of a commercial coagulometer and capillary viscometer. The results showed that the frequency shift caused by blood coagulation is greatly enhanced and more than twice that of a traditional QCM. Also, a higher reaction temperature not only accelerates the coagulation reaction but also weakens the frequency response. A comparison between the plasma viscosity measured with the QCM and that measured with the capillary viscometer shows that the QCM can measure plasma viscosity well on the basis of the theory of the liquid attenuation layer. Our research indicates that QCM-based coagulation measurement can not only measure the coagulation time but also the plasma viscosity, which is meaningful for related fields.

1. Introduction

Blood transports nutrients and resists external infections and this is a very important part of the human body. The blood coagulation function plays a very important role when there is damage inside or outside the body; some blood quickly changes from liquid phase to solid phase to block the damaged area to prevent blood loss and infection. Therefore, the blood coagulation time is an important index. For some patients with coagulation-related diseases such as vascular disease,⁽¹⁾ regular monitoring of the coagulation time is necessary. After certain surgical procedures, it is also necessary to control the coagulation time with drugs.⁽²⁾ Currently, the measurement methods employed in coagulometers widely used in hospitals and other institutions

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<https://doi.org/10.18494/SAM3774>

are based on optics and magnetoelasticity,^(3,4) but these instruments are bulky and expensive. Although large-scale coagulometers with excellent performance can simultaneously measure multiple samples, patients must visit the hospital regularly for coagulation tests, making them time-consuming and unfeasible for busy people. Therefore, there is an urgent need for a fast but inexpensive, portable detection method to measure coagulation time.

The quartz crystal microbalance (QCM) is a piezoelectric sensor that can detect mass change,^(5–9) liquid viscosity and density,^(10,11) and interfacial tension,^(12,13) and has been widely applied in many fields such as chemistry,^(14–16) the environment,^(17,18) and especially biomedicine.^(19–22) Its detection limit can reach nanogram level, but the testing instrument is simple and inexpensive, and real-time detection is also its advantage. The QCM originated from the Sauerbrey equation derived by Sauerbrey in 1959,⁽²³⁾ which shows that the relationship between the resonant frequency shift and mass change adhered to the surface of the QCM wafer is linear. In the 1980s, Kanazawa and Gordon proposed an equation showing the relationship between the resonant frequency shift and the density and viscosity of a liquid on the surface of the QCM wafer. The Kanazawa–Gordon equation^(10,24) is

$$\Delta f = -f_0^{3/2} \left(\eta \rho / (\pi \mu_q \rho_q) \right)^{1/2}, \quad (1)$$

where f_0 is the fundamental frequency of the QCM, η and ρ are the liquid viscosity and density, and μ_q and ρ_q are the shear modulus and density of quartz crystal, respectively. It can be found from the Kanazawa–Gordon equation that the frequency shift has a linear relationship with the square root of the product of the liquid density and viscosity. During the coagulation process, the plasma gradually changes from liquid to gelatinous substance. Therefore, the viscosity of the liquid changes dramatically, and this change can be sensed by a QCM, making it possible to use a QCM to measure the coagulation time.

From the Kanazawa–Gordon equation, the QCM sensitivity to liquid density and viscosity is significantly influenced by the fundamental frequency of the QCM. Commonly used QCM frequencies are 5 and 10 MHz, and according to our experiments in this study, we found that the frequency response of 10 MHz QCM to blood coagulation is about 790 Hz. The plasma was undiluted standard plasma, and the reagent was also undiluted; therefore, the corresponding blood coagulation effect should be good. Similar results have been reported in Refs. 25–27, in which the frequency shift caused by blood coagulation was about 300–800 Hz. To enhance the frequency response of the QCM to blood coagulation, we can appropriately increase the fundamental frequency of the QCM, but the frequency cannot be increased too much because of the damping on the QCM wafer caused by the plasma and reagent.^(28,29) Another approach is to modify the electrode to obtain higher mass sensitivity.

Kanazawa, Gordon, and Martin,^(10,24,30) attributed the ability of the QCM to detect the liquid density and viscosity to the liquid attenuation layer adjacent to the QCM surface. The equation for the thickness of the attenuation layer is^(10,24)

$$\delta = \sqrt{\frac{\eta}{\pi f_0 \rho}}. \quad (2)$$

Here, δ is the thickness of the liquid attenuation layer, and the thickness of the effective mass layer is $\delta/2$. f_0 is the fundamental frequency of the QCM, and η and ρ are the liquid viscosity and density, respectively. Therefore, it is the mass of the liquid effective mass layer that causes the change in the QCM frequency.⁽³¹⁾ Note that not only liquid density, viscosity, and QCM frequency affect the thickness of the liquid attenuation layer, but surface roughness and hydrophobicity also have influence.^(11,32–35) In our previous research,^(36–38) we found that the QCM mass sensitivity is affected by the diameter and thickness of the electrode and also by the electrode structure. For the commonly used circular electrode, the mass sensitivity at the center of the QCM wafer does not change monotonically with the electrode diameter but there is a maximum point, and it also changes with electrode thickness.^(37,38) Therefore, it is feasible to improve the QCM frequency response to blood coagulation by modifying the electrode structure and size.

In this study, after careful consideration, a fundamental frequency of 13 MHz, an n-m-type electrode, and a specific electrode size were adopted, and the thrombin time (TT) was employed to verify the improvement. The effect of the reaction temperature on TT measured with the QCM was experimentally verified and compared with that measured with a commercial coagulometer. The experiment verified that it is feasible to measure plasma viscosity on the basis of the theory of the liquid attenuation layer.

2. Experimental Procedure

2.1 Reagents and samples

The standard plasma and TT reagent were provided by Chengdu Excellent Medical Scientific Co., Ltd (Chengdu, China). All reagents and samples were refrigerated before use.

2.2 Instrumentation

S&AW-5600 base plating system (Saunders & Associates, LLC, Phoenix, USA) was provided by Wintron Electronic Company, Ltd. (Zhengzhou, China). A micropipette (SL-100XLS, METTLER TOLEDO, Switzerland), a commercial coagulometer (AKSL EC6800, Chengdu Excellent Medical Scientific), and a water bath (DF-101, LICHENKEYI, China) were provided by Chengdu Excellent Medical Scientific. A capillary viscometer was purchased from Shanghai Glass Instrument Factory. Vector network analyzer (NanoVNA-H) was purchased online, and its frequency stability is 0.3 ppm.

2.3 QCM

The QCMs used in this study were manufactured in a class 10000 ultraclean room of Wintron Electronic Company, Ltd. (Zhengzhou, China). The fundamental frequency of each QCM was about 13.06 MHz. The surface of the quartz wafer was polished, then silver electrodes were plated on both sides by physical vapor deposition. The obtained QCMs were inspected visually,

and those without defects were packaged. The electrode type was n-m, that is, the electrodes were circular but the diameters of the electrodes on both sides of the quartz wafer were different. The diameter of the quartz crystal wafer was 8 mm and those of the electrodes were 4.5 and 2.5 mm; the electrode thickness was 150 nm.

For the commonly used circular electrode, the mass sensitivity at the electrode center of the QCM wafer does not change monotonically with the electrode diameter but there is a maximum point.^(37,38) This phenomenon is also true for the n-m-type electrode, and in our previous work,⁽³⁶⁾ we found that the mass sensitivity was maximum for a 10 MHz QCM with electrode diameters on opposite sides of 2.5 and 5.1 mm. While for a 13 MHz QCM in this study, the electrode diameters to obtain the maximum mass sensitivity were 2.5 and 4.5 mm.

The mass sensitivity is also influenced by the electrode thickness; within a certain range of electrode thickness, the thicker the electrode, the higher the sensitivity.⁽³⁷⁾ However, if the electrode is too thick, the Q value drops sharply. The selected thickness in this study was slightly higher than the commonly used thickness (100 nm), and experiments in our previous work⁽³⁹⁾ verified that the mass sensitivity was improved to a certain extent. Moreover, the Q value did not change significantly compared with that of the electrode thickness of 100 nm. Therefore, an electrode thickness of 150 nm was selected in this study.

2.4 TT measurement by QCM

The QCM was driven by a VNA (vector network analyzer) connected to a computer. The frequency of minimum attenuation (S21) is calculated and displayed by laboratory-made software. The experiment was carried out in an ordinary room of Chengdu Excellent Medical Scientific whose ambient temperature of the room was maintained at about 25 °C. The experimental setup is shown in Fig. 1. The QCM connected to the VNA is placed in an incubator. A temperature sensor in the incubator is used to ensure that the temperature around the QCM is right (28 and 40 °C, respectively). Before the experiment, all the plasma and reagents were removed from the refrigerator and placed in a water bath at 28 or 40 °C for about 4 min. After the QCM was connected to the VNA and the frequency was stable, 15 µl of plasma was dropped at the center of the surface of the QCM wafer through a micropipette. Then when the frequency was stable, 15 µl of TT reagent was dropped to the plasma droplet. After they reacted for about 150 s, the frequency did not decrease further. We carried out three measurements each at the two reaction temperatures (28 and 40 °C).

2.5 TT measurement by coagulometer

For a comparison with the QCM, we used an AKSL EC6800 commercial coagulometer, whose detection method is magnetic bead vibration. The standard reaction temperature of the AKSL EC6800 coagulometer is about 40 °C, and it can be set to non-heating mode with a corresponding reaction temperature of about 28 °C. To minimize the experimental error, the coagulometer measurements were carried out simultaneously with the QCM measurements.

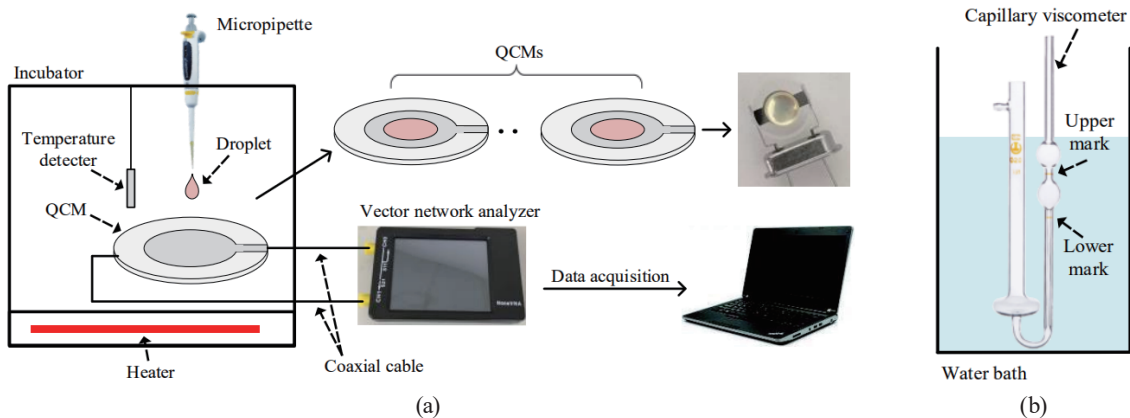


Fig. 1. (Color online) Schematic of the experimental setup. (a) Coagulation process measured with the QCM. (b) Viscosity of glycerol/water solutions and plasma measured with the capillary viscometer.

2.6 QCM mass sensitivity calibration and plasma viscosity measurement using capillary viscometer

A capillary viscometer was used to accurately obtain the viscosity of the glycerol/water solution at 28 °C and plasma at 28 and 40 °C. As shown in Fig. 1(b), the capillary viscometer was placed in the water bath, and a sample with a volume of about 6 ml was placed in the capillary viscometer. After about 7 min, the upper liquid level of the sample was moved to the upper mark using a rubber suction bulb. Then the liquid was allowed fall freely along the capillary under the action of gravity and timing was started. The timing was stopped when the upper liquid level of the sample reached the lower mark. We carried out three measurements each at the two reaction temperatures (28 and 40 °C). Note that the viscosity measured with the capillary viscometer is kinematic viscosity, the viscosity for the Kanazawa–Gordon equation is dynamic viscosity, and the viscosity conversion equation is (dynamic viscosity = kinematic viscosity × liquid density).

The glycerol/water solution is used to calibrate the QCM mass sensitivity on the basis of the theory of the liquid attenuation layer. A certain amount of glycerol/water solution was dropped to the center of the surface of the QCM wafer, and the radius of the region covered by the solution was made as close as possible to the radius of the region covered by 15 µl of plasma. After the frequency stabilized, the frequency shift was recorded. The temperature of the glycerol/water solution and incubator was 28 °C. Then the equivalent mass sensitivity of the region covered by samples was calculated as follows.

$$S_E = \frac{\Delta f}{\Delta m} = \frac{2\Delta f}{\pi r^2 \delta \rho} \quad (3)$$

Here, r is the radius of the region covered by the sample, S_E is the equivalent mass sensitivity of this region, Δm is the mass of the effective mass layer, Δf is the frequency shift caused by the glycerol/water solution at 28 °C, and ρ is the density of the glycerol/water solution. The concentrations of the glycerol/water solutions are 0, 10, 20, 30, 40, and 50% (glycerol weight %).

3. Results and Discussion

The frequency change of the QCM during the coagulation process is shown in Fig. 2. The first sharp frequency decrease is due to the first droplet of 15 μl of plasma on the QCM wafer surface, after which the frequency quickly stabilized. When 15 μl of TT reagent was added to the droplet of plasma, the two droplets mixed quickly and the frequency of the QCM began to decrease. Note that there is a large jitter in the frequency after the addition of the TT reagent; this is caused by the droplet impacting on the QCM wafer surface and the manual operation.

As shown in Fig. 2(a), during the initial reaction, the frequency decreases slowly, but the rate of decrease also increases. After the reaction has continued for a period of time, the rate of decrease reaches a maximum, which is retained before the rate of decrease begins to decrease until the reaction stops. The slow reaction rate of the coagulation during the initial period may be due to the mixing of the plasma and reagent. However, this phenomenon is not always obvious. Most of the time, the mixing of the plasma and the reagent is complete after the reagent is added; the coagulation reaction rate quickly reaches maximum, which is retained for a period of time, and then the reaction rate slowly decreases until the reaction stops. The entire reaction process is consistent with the reaction mechanism of TT. That is, after adding the reagent, thrombin directly converts fibrinogen into fibrin. In Figs. 2(b), 2(c), and 2(e), there is a sudden increase in frequency when the coagulation process is about to end, and then the frequency rapidly decreases to its previous value.⁽⁴⁰⁾ This may have been caused by the clot falling off the

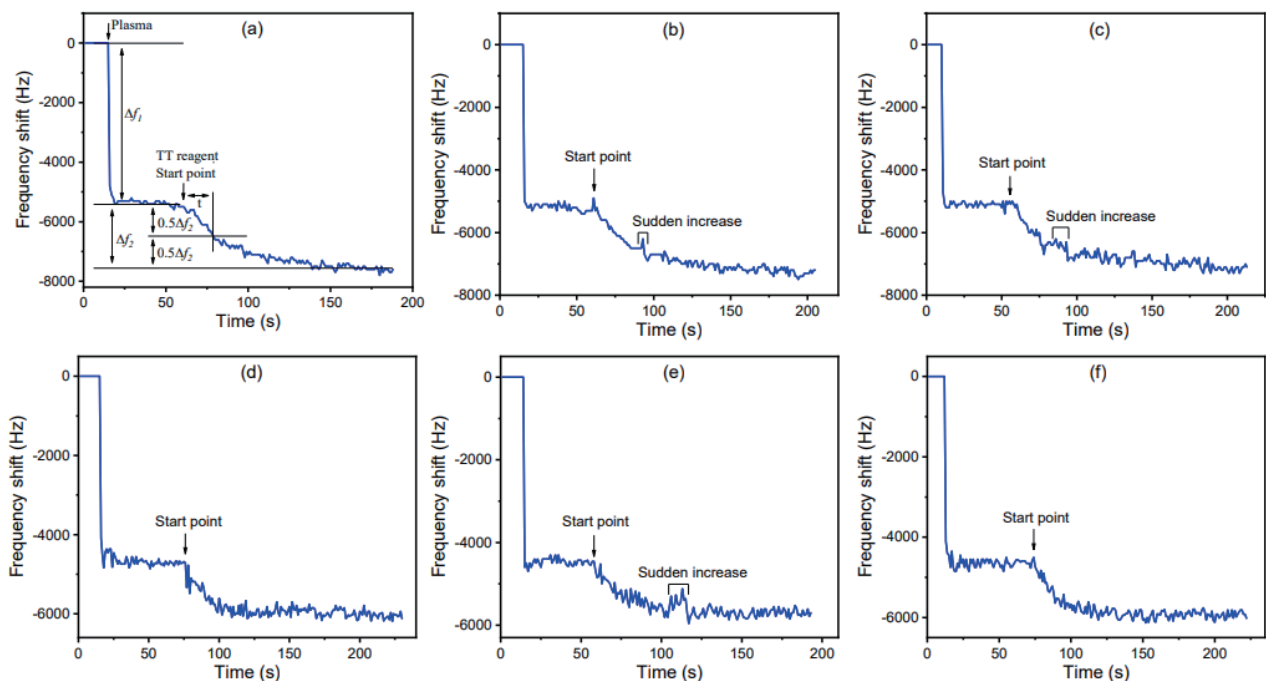


Fig. 2. (Color online) QCM frequency shifts of standard plasma during coagulation process. The method of determining the coagulation time is shown in (a). The reaction temperatures of (a)–(c) and (d)–(f) are 28 and 40 °C, respectively.

electrode surface,⁽⁴⁰⁾ in such a case, the clot was quickly adhered to the electrode surface again. In addition, this phenomenon cannot always be detected.

3.1 Plasma viscosity

The glycerol/water solution was used to calibrate the equivalent mass sensitivity of the region covered by the plasma. The corresponding results are shown in Table 1. η_C is the glycerol/water solution viscosity measured with the capillary viscometer. Δf is the QCM frequency shift caused by the droplet of the glycerol/water solution, and $\overline{\Delta f}$ is the corresponding average value. From these values, the equivalent mass sensitivity (S_E) was calculated and shown in Table 1. $\overline{S_E}$ is the average value of S_E . Then, from Eqs. (2) and (3), the expression of plasma viscosity can be obtained as

$$\eta_Q = \frac{4\Delta f_1^2 f_0}{\pi r^4 \rho_p S_E^2}, \quad (4)$$

where ρ_p is the density of the plasma; here, we use a value of 1030 kg/m³.

The results for plasma temperatures of 28 and 40 °C are shown in Table 2. In the table, r is the radius of the region covered by the plasma. Δf_1 is the frequency shift caused by the plasma, as shown in Fig. 2(a). $\overline{\Delta f_1}$ and RSD are the average value and relative standard deviation of Δf_1 , respectively, η_C is the plasma viscosity measured with the capillary viscometer. η_Q is the plasma viscosity obtained with the QCM, and E_η is the error between η_C and η_Q .

Table 1
Equivalent mass sensitivity calibration.

Glycerol/water (Wt %)	0	10	20	30	40	50
η_C (mPa·s)	0.844	1.068	1.435	1.926	2.799	4.64
Density (g/ml)	1	1.0267	1.0527	1.0793	1.1	1.12
Δf (Hz)	4037 / 3883 / 3930	4168 / 4184 / 4277	5030 / 5048 / 5101	6196 / 6166 / 6343	7580 / 7816 / 7880	10148 / 10084 / 10035
$\overline{\Delta f}$ (Hz)	3950	4210	5060	6235	7759	10089
S_E (Hz/ng)	4.38	4.1	4.2	4.41	4.51	4.51
$\overline{S_E}$ (Hz/ng)	4.35					

Table 2
Plasma viscosity.

Temperature (°C)	28	40
r (mm)	2	2
Δf_1 (Hz)	5312 / 5225 / 5173	4707 / 4479 / 4617
$\overline{\Delta f_1}$ (Hz)	5236.7	4601
RSD (%)	1.34	2.5
η_Q (mPa·s)	1.46	1.13
η_C (mPa·s)	1.429	1.1
E_η (%)	2.2	2.7

From Table 2, we can find that the QCM frequency shifts caused by blood coagulation at 28 and 40 °C are different. Within a certain range, the temperature does not have a significant effect on the plasma density but has a significant effect on the viscosity. Therefore, the difference is mainly caused by the change in the plasma viscosity with the temperature. The low E_η indicates the feasibility of measuring plasma viscosity using the QCM on the basis of the theory of the liquid attenuation layer.

3.2 TT

After the addition of the TT reagent, the coagulation process starts, and the QCM frequency starts to decrease. The coagulation reaction ends when the frequency no longer decreases. However, it is difficult to determine the end point of the coagulation reaction from this observation. Owing to the low quality factor caused by high damping, the frequency stability is much lower in the liquid phase than in the gas phase.^(28,29) Therefore, judging the end of the coagulation on the basis of the no longer decreasing frequency may cause a large error. During the coagulation, the frequency mainly decreases. Therefore, we adopt a method in which the end point of coagulation is defined as the point when the frequency first reaches $\Delta f_2/2$ as shown in Fig. 2(a).

The results are shown in Table 3. In the table, t is the coagulation time obtained with the QCM and coagulometer, and Δt and RSD are the average value and relative standard deviation of t , respectively. From Table 3, we find that the RSD of the coagulometer is very low, indicating the very high reliability of the commercial coagulometer. From TT measured with the coagulometer, we find that the reaction temperature affects the coagulation time, and TT at 40 °C is about 10% lower than that at 28 °C, indicating that a higher reaction temperature accelerates the coagulation reaction. For the QCM, TT at 40 °C is about 40% lower than that at 28 °C. The acceleration effect for the QCM is significantly higher than that for the coagulometer. However, the measurement method is unlikely to influence the effect of the reaction temperature on the coagulation reaction. The larger difference in TT measured with the QCM between 28 and 40 °C may be related to the measurement method and the end point determination method. Also, the measurement method and end point determination method also affect the difference in TT between the QCM and the coagulometer.

3.3 Sensitivity enhancement

The frequency shift caused by coagulation is denoted as Δf_2 , as shown in Fig. 2(a). A comparison between Δf_2 for traditional QCMs^(25–27) and that in this study is shown in Fig. 3. We

Table 3
TT.

	Temperature (°C)	QCM		Coagulometer	
		28	40	28	40
Standard plasma	t (s)	24 / 26 / 24	14 / 14 / 16	22 / 22.6 / 22.3	20.2 / 19.9 / 19.8
	Δt (s)	24.7	14.7	22.3	20
	RSD (%)	4.7	7.87	1.3	1

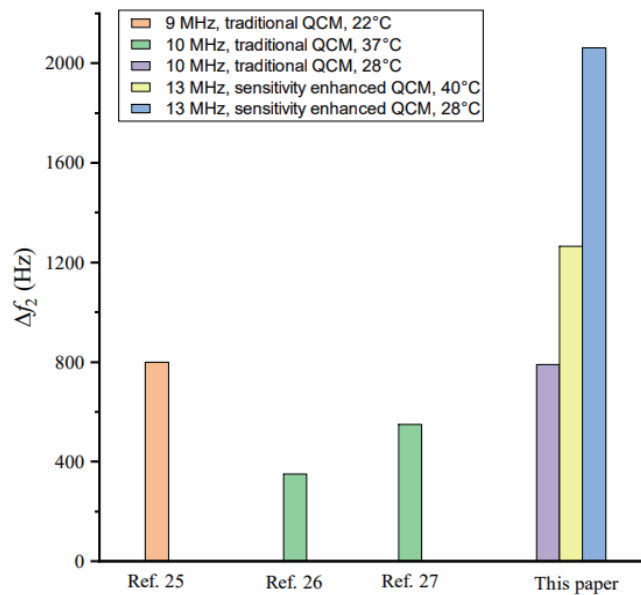


Fig. 3. (Color online) Comparison between the frequency shift caused by coagulation for traditional QCM and sensitivity-enhanced QCM.

find that the frequency shift (Δf_2) caused by coagulation is greatly enhanced in this study, and is more than twice that for traditional QCMs,^(25–27) indicating the large improvement of the frequency response to blood coagulation. In addition, comparing the frequency shift (Δf_2) at 28 and 40 °C, we find that the frequency shift (Δf_2) at 40 °C is about 60% of that at 28 °C, which may be due to the higher temperature and faster coagulation reaction at 40 °C.⁽⁴¹⁾

4. Conclusions

To enhance the QCM frequency response caused by blood coagulation and analyze the applicability of the QCM in plasma viscosity and coagulation measurements, a mass-sensitivity-enhanced QCM is designed. Our experimental results verified that our specially designed QCM can greatly improve the frequency response to blood coagulation, which is conducive to the application of QCMs in coagulation measurement. A comparison between results obtained using the QCM and a coagulometer shows that the QCM is fully capable of measuring the coagulation process and the coagulation time. Values of TT obtained with the QCM and a commercial coagulometer at different reaction temperatures indicate that a higher reaction temperature accelerates the coagulation reaction. A comparison between the plasma viscosity obtained with the QCM and that obtained with a capillary viscometer shows that it is feasible to measure the plasma viscosity on the basis of the theory of the liquid attenuation layer. The frequency drop curve can closely reflect the coagulation process, which is of great significance for monitoring biological reaction processes through density and viscosity. Furthermore, owing to the merits of portability, simple operation, and the capability of real-time detection, the QCM is an excellent coagulation measurement tool, especially for the development of portable coagulation instruments.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 61871098).

References

- 1 R. A. Marlar, B. Clement, and J. Gausman: *Semin. Thrombosis Hemostasis* **43** (2017) 253. <https://doi.org/10.1055/s-0036-1581128>
- 2 J.-P. Francoia, R. Pascal, and L. Vial: *Chem. Commun.* **51** (2015) 1953. <https://doi.org/10.1039/C4CC08563A>
- 3 L. F. Harris, V. Castro-López, and A. J. Killard: *TrAC, Trends Anal. Chem.* **50** (2013) 85. <https://doi.org/10.1016/j.trac.2013.05.009>
- 4 K. M. Hansson, T. P. Vikinge, M. Rånby, P. Tengvall, I. Lundström, K. Johansen, and T. L. Lindahl: *Biosens. Bioelectron.* **14** (1999) 671. [https://doi.org/10.1016/S0956-5663\(99\)00050-0](https://doi.org/10.1016/S0956-5663(99)00050-0)
- 5 V. M. Mecea: *Sens. Actuators, A* **128** (2006) 270. <https://doi.org/10.1016/j.sna.2006.01.023>
- 6 F. Josse, Y. Lee, S. J. Martin, and R. W. Cernosek: *Anal. Chem.* **70** (1998) 237. <https://doi.org/10.1021/ac9706032>
- 7 W. Pan, X. H. Huang, and Q. Chen: *IEEE. Trans. Ultrason. Ferroelectr. Freq. Control* **67** (2020) 1953. <https://doi.org/10.1109/TUFFC.2020.3008790>
- 8 X. H. Huang, Q. S. Bai, J. G. Hu, and D. Hou: *Sensors* **17** (2017) 1785. <https://doi.org/10.3390/s17081785>
- 9 Q. Chen, X. H. Huang, W. Pan, and Y. Yao: *Chin. Phys. Lett.* **36** (2019) 120702. <https://doi.org/10.1088/0256-307X/36/12/120702>
- 10 K. K. Kanazawa and J. G. Gordon: *Anal. Chem.* **57** (1985) 1770. <https://doi.org/10.1021/ac00285a062>
- 11 L. A. Theisen, S. J. Martin, and A. R. Hillman: *Anal. Chem.* **76** (2004) 796. <https://doi.org/10.1021/ac034777x>
- 12 Z. X. Lin and M. D. Ward: *Anal. Chem.* **68** (1996) 1285. <https://doi.org/10.1021/ac951131z>
- 13 D. A. Buttry and M. D. Ward: *Chem. Rev.* **92** (1992) 1355. <https://doi.org/10.1021/cr00014a006>
- 14 Q. Chen, Y. Yao, X. H. Huang, D. Liu, and K. L. Mao: *Sens. Actuators, B* **341** (2021) 129992. <https://doi.org/10.1016/j.snb.2021.129992>
- 15 J. Wang, S. H. Lin, Y. L. Lin, and X. H. Wang: *Sens. Mater.* **33** (2021) 499. <https://doi.org/10.18494/SAM.2021.3075>
- 16 K. Noda, H. Aizawa, and K. Marumoto: *Sens. Mater.* **32** (2020) 2159. <https://doi.org/10.18494/SAM.2020.2877>
- 17 J. Lee, M. Atmeh, and D. Berman: *Carbon* **120** (2017) 11. <https://doi.org/10.1016/j.carbon.2017.05.008>
- 18 D. Q. Chen, X. Y. Sun, K. H. Zhang, G. K. Fan, Y. Wang, G. Li, and R. F. Hu: *Sensors* **17** (2017) 1681. <https://doi.org/10.3390/s17071681>
- 19 L. Yang, X. H. Huang, L. Sun, and L. Xu: *Sens. Actuators, B* **224** (2016) 863. <https://doi.org/10.1016/j.snb.2015.11.002>
- 20 K. D. Esmeryan, R. R. Ganeva, G. S. Stamenov, and T. A. Chaushev: *Sensors* **19** (2019) 123. <https://doi.org/10.3390/s19010123>
- 21 K. D. Esmeryan, G. S. Stamenov, and T. A. Chaushev: *Sens. Actuators, A* **297** (2019) 111532. <https://doi.org/10.1016/j.sna.2019.111532>
- 22 K. D. Esmeryan and T. A. Chaushev: *Sens. Actuators, A* **317** (2021) 112480. <https://doi.org/10.1016/j.sna.2020.112480>
- 23 G. Sauerbrey: *ZPhy* **155** (1959) 206. <https://doi.org/10.1007/BF01337937>
- 24 K. K. Kanazawa and J. G. Gordon: *Anal. Chem. Acta* **175** (1985) 99. [https://doi.org/10.1016/S0003-2670\(00\)82721-X](https://doi.org/10.1016/S0003-2670(00)82721-X)
- 25 G. Guhr, R. Kunze, G. Martin, H. Schmidt, M. Weihnacht, S. Gehrisch, and G. Siegert: *IEEE Ultrasonics Symp.* (2006) 544. <https://doi.org/10.1109/ULTSYM.2006.141>
- 26 L. Muller, S. Sinn, H. Drechsel, C. Ziegler, H.-P. Wendel, H. Northoff, and F. K. Gehring: *Anal. Chem.* **82** (2009) 658. <https://doi.org/10.1021/ac9021117>
- 27 M. Hussain, K. Schmidt, M. K. Körber, E. Langer, O. Faul, H. Northoff, H. P. Wendel, C. von Heymann, and F. K. Gehring: *Sens. Actuators, B* **275** (2018) 110. <https://doi.org/10.1016/j.snb.2018.08.049>
- 28 L. Rodriguez-Pardo, J. F. Rodríguez, C. Gabrielli, H. Perrot, and R. Brendel: *IEEE Sens. J.* **5** (2005) 1251. <https://doi.org/10.1109/JSEN.2005.859257>
- 29 M. Rodahl, F. Höök, A. Krozer, P. Brzezinski, and B. Kasemo: *Rev. Sci. Instrum.* **66** (1995) 3924. <https://doi.org/10.1063/1.1145396>
- 30 S. J. Martin, V. E. Granstaff, and G. C. Frye: *Anal. Chem.* **63** (1991) 2272. <https://doi.org/10.1021/ac00020a015>

- 31 Q. S. Bai and X. H. Huang: *Sens. Mater.* **29** (2017) 539. <https://doi.org/10.18494/SAM.2017.1434>
- 32 P. Roach, G. McHale, C. R. Evans, N. J. Shirtcliffe, and M. I. Newton: *Langmuir* **23** (2007) 9823. <https://doi.org/10.1021/la701089a>
- 33 G. McHale, P. Roach, C. R. Evans, N. J. Shirtcliffe, S. J. Elliott, and M. I. Newton: *IEEE Int. Frequency Control Symp.* (2008) 698. <https://doi.org/10.1109/FREQ.2008.4623089>
- 34 W. C. Duncan-Hewitt and M. Thompson: *Anal. Chem.* **64** (1992) 94. <https://doi.org/10.1021/ac00025a017>
- 35 M. Yang, M. Thompson, and W. C. Duncan-Hewitt: *Langmuir* **9** (1993) 802. <https://doi.org/10.1021/la00027a033>
- 36 Q. Chen, X. H. Huang, W. Pan, Y. Xu, and Z. C. Fan: *Sensors* **19** (2019) 2125. <https://doi.org/10.3390/s19092125>
- 37 W. Pan, X. H. Huang, Q. Chen, Z. C. Fan, and Y. Xu: *Chin. Phys. Lett.* **36** (2019) 070701. <https://doi.org/10.1088/0256-307x/36/7/070701>
- 38 X. H. Huang, W. Pan, J. G. Hu, and Q. S. Bai: *IEEE. Trans. Ultrason. Ferroelectr. Freq. Control* **65** (2018) 1888. <https://doi.org/10.1109/TUFFC.2018.2860597>
- 39 X. H. Huang, Q. Chen, W. Pan, and J. G. Hu: *IEEE. Trans. Ultrason. Ferroelectr. Freq. Control* **68** (2020) 1458 <https://doi.org/10.1109/TUFFC.2020.3030636>
- 40 C. Yao, L. Qu, and W. Fu: *Sensors* **13** (2013) 6946. <https://doi.org/10.3390/s130606946>
- 41 D. Chen, S. R. Song, J. L. Ma, Z. Zhang, P. Wang, W. H. Liu, and Q. Q. Guo: *Biosens. Bioelectron.* **91** (2017) 465. <https://doi.org/10.1016/j.bios.2016.12.063>

