OSCILLATION FREQUENCY STABILITY OF QCM-BIOSENSOR IN LIQUID BASED ON ELECTRONIC CIRCUIT TECHNOLOGY FOR TELECOMMUNICATION

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Abstract－The frequency stability of QCM is one of the important parameters for realizing a high sensitivity biosensor for dioxin, PCB and marker protein. However, in the research of QCM, the frequency measuring method focused on quartz crystal sensors has not been fully examined yet. In this paper, an experiment system for such method is proposed.

Ⅰ. INTRODUCTION

We have developed a practical biosensor based on crystal resonator technology. The sensor exploits immune reactions to recognize target analysis. Mass loading affects the quartz crystal resonance which can be monitored. The change in the resonant frequency of the crystal oscillator is proportional to bio-specific mass adsorption. This sensor generally measures immune reactions in a liquid. In the liquid, the Q factor of the sensor can be equal to or less than 5,000. There are reports of the use of digital IC and impedance analysis for QCM interrogation. Using a 9 MHz QCM with such Q factor, measurement down to 1Hz precision is difficult mainly due to electronic noise in frequency vs. phase of the crystal [1] [2]. In QCM, we therefore average frequency measurements to minimize this problem. However, by solving this problem, we will get closer to more practical uses of QCM.

Ⅱ. PURPOSE

Using radio telecommunication technology, we propose a new QCM system and new quartz crystal sensor. In this system, the radio telecommunication technology is applied to this circuit such that the noise and the minute signal are considered. Short-term frequency stability of the order of 10^{-9} can be established. An AT-cut 9MHz QCM of high sensitivity is developed. In liquid, the oscillation of the sensor exhibits high stability. In PBS buffer liquid, we can achieve long-term frequency stability (over 1 hour) of less than 0.1ppm. We studied oscillation frequency deviation vs. concentration of antibody in liquid.

Ⅲ. MEASUREMENT METHOD QCM

Using a network analyzer or impedance analyzer, if the Q factor of quartz crystal resonator becomes low (2,000 or less), and the resonance frequency measurement accuracy of the analyzer decreases (maybe 4Hz/0.2degree on 9MHz). On the other hand, when the series resistance of quartz crystal resonator is changed, measurement accuracy of resonance frequency with series resistance reduces the performance [4] [5]. Therefore, when the Q factor is low, the “resonance frequency vs. phase” change is subject to large variations, and sensitivity deteriorates. Ultimately, impedance matching cannot be realized. Therefore, use of such analyzers for QCM becomes impractical. When a digital IC is used for an oscillating circuit as for QCM, it is necessary to compensate for electric noise when making measurements [3]. We propose an ANALOG oscillating circuit based on telecommunication technology for QCM operations

Ⅳ. EXPERIMENT SYSTEM

A. Evaluation Environment

We have adopted the flow injection technique for QCM bio-sensor system. It shows the figure.1. This is why the quartz crystal sensor’s response must be stable. To complete our system, a syringe pump and injector are required. Flow cell system allows for crystal sensor assembly. The flow cell volume is approx. 0.35mL. In all cases, the flow rate must be kept at around 20mL/min.

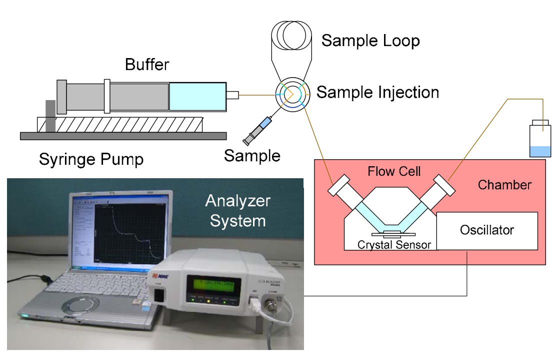

Fig. 1 Experimental system QCM

B. Quartz Crystal Sensor

Quartz crystal resonator is 9MHz of AT cut fundamental vibration mode. The surface of the crystal blank is polish process to keep it flat. Electrode is gold Drive level is fixed 0.3mA±0.01mA in every conditions against DLD issue.[6] Quartz crystal sensor shows fig.2.

- Frequency : 9MHz、AT cut
- Mode : Fundamental Vibration Mode
- Surface : Polishing
- Electrode : Gold Plate on Chromium
- Structure : Laguban type

Fig.2 Quartz Crystal Sensor

C. Oscillator Circuit For QCM Analyzer

Generally, the mechanical Q factor in liquid is 5000 or less. Oscillator based on digital IC is used by QCM field and frequency stability is issued by electronic noise. This signal noise is influenced by short term stability. For oscillation frequency stability of QCM, we focused on the signal noise based on electric circuit technology for telecommunication, especially on the phase noise characteristic. In this experiment system, the crystal oscillator based on the colpitts is used which can secure a frequency stability of 0.1ppm. Preventive measures against electric noise in oscillator are taken in the measurement system for QCM, paying particular attention to phase noise characteristics.

The phase noise characteristic in liquid condition is showed the figure.3 by compared oscillator based on the low phase noise type.

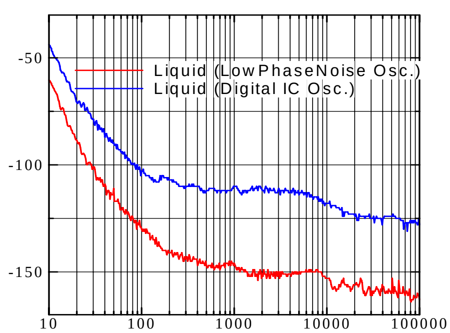

Fig.3 Phase noise characteristic

D. Analyzer Block Diagram

The Analyzer mainly consists of analog and digital logic. It consists of oscillator circuits. Heterodyne detection is carried out by the AD-converter, and it changes into a discrete digital signal. As for digital signal after conversion, digital signal processing of a phase differential system is performed.

A low phase noise type OCXO is used as a signal source to generate standard clock. Fig.4 shows the analyzer block diagram.

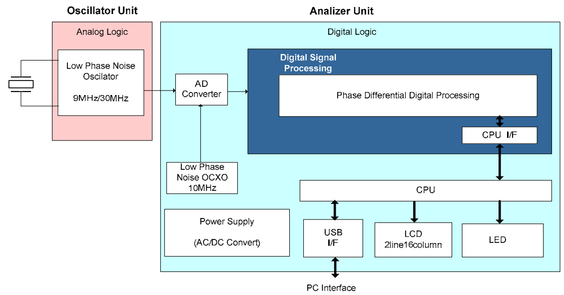

Fig.4 Analyzer block diagram

V. RESULT

A. Frequency Stability

The short-term stability in liquid environment is below 0.01ppm (0.09Hz) per second. When antibody is attached to the crystal sensor, the sensor response shows long term stability of 0.05ppm over 1.5hrs (0.45Hz).

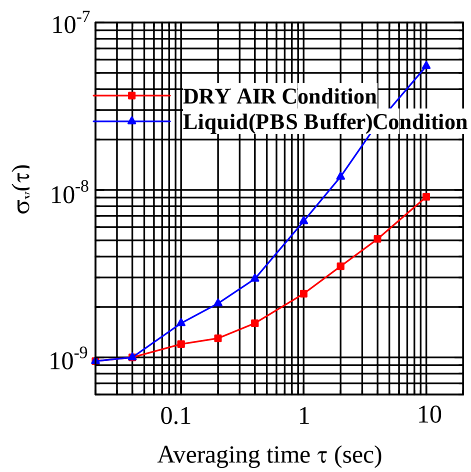

Fig.5 Measurement example of short-term frequency stability

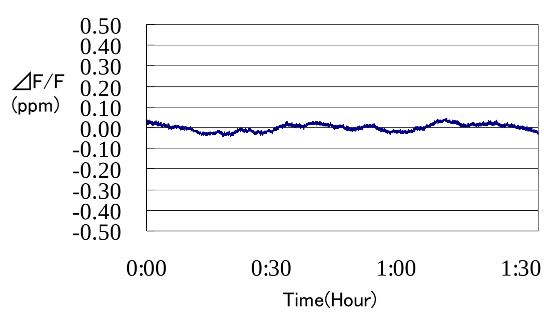

Fig. 6 Measurement example of log-term frequency stability in liquid

B. Immune reaction

With using this system, we inspected the propriety of our system by immune reaction.

(1)Results of CRP monochrome reaction

As fig.7 shows, CRP antibody is immobilized to electrode of quartz crystal resonator, BSA protein is blocked to gap among CRP antibody. Anti-CRP antibody is applied and the antibody concentration dependence of oscillation frequency is examined.

Frequency state of reaction shows Fig.8.that it's the frequency shift in correspondence with a molecule mass. Fig.9 shows a measurement sample of CRP antibody concentration. The concentration of the immune antibody, anti-CRP, is 0.1μ g/mL, 1μg/mL, 10μg/mL and 100 μ g/mL. The frequency change due to the antigen-antibody reaction is 0.03ppm to 6.2ppm (0.27Hz to 55.8Hz). We were confirmed that the amount of oscillation frequency shift corresponding to the concentration density of anti-CRP monochrome.

From these results, we confirm that the design condition of the crystal sensor experiment system can be used for QCM bio-sensing.

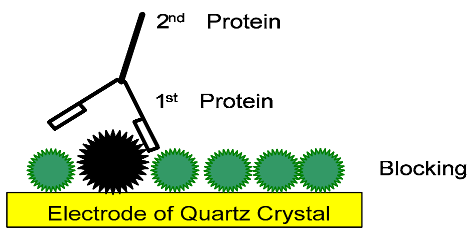

Fig.7 Immobilized CRP Protein

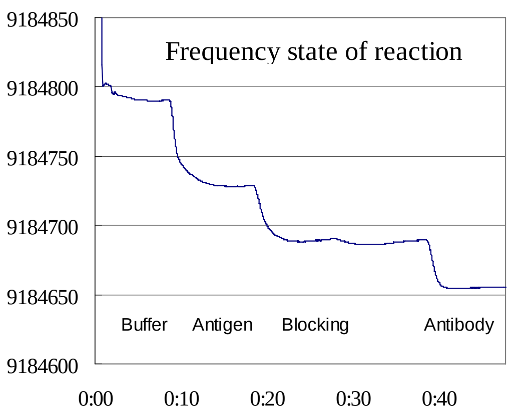

Fig.8 Frequency state of reaction

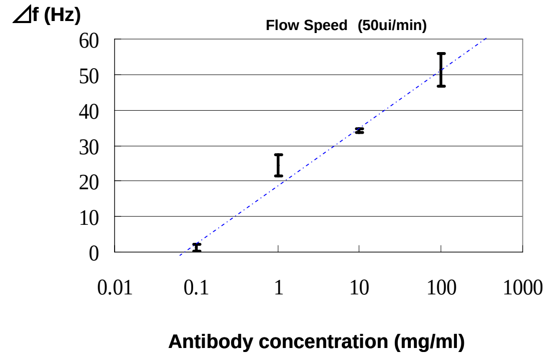

Fig.9 Measurement Sample of CRP antibody concentration

(2)Results of Human IgG reaction

As fig.10 shows, Anti-Human IgG is applied as antibody. Fig.11 shows a measurement sample of Human IgG concentration. The concentration density slope of immune reaction of Human IgG is more subtle due to the lower molecular weight in comparison with anti-CRP. This 9MHz crystal resonator result demonstrates the measuring possibility range can be expanded 10ng/ml density.

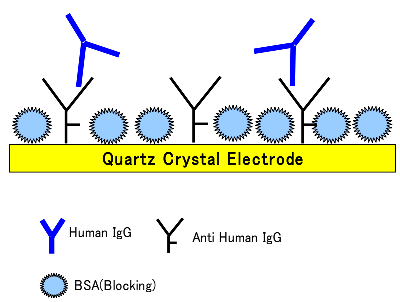

Fig.10 Immobilized Human IgG Protein

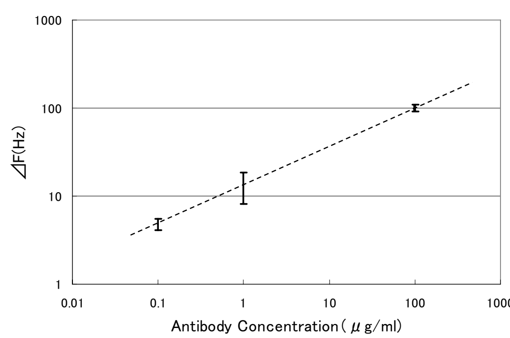

Fig11. Measurement Sample of Human IgG concentration

(3)Corresponding Molecular weight

We consider the frequency change of reaction for some chemical reagents with varying molecular weight all at 100μg/mL. We see that the chemical reagents with higher molecular weight result in a larger frequency shift of reaction. It shows Fig.12.

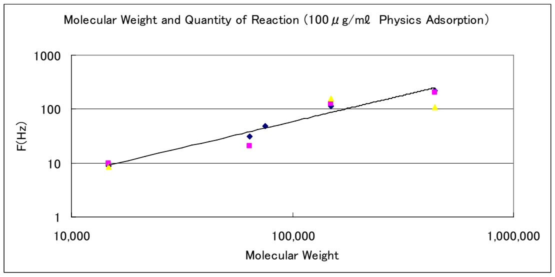

Fig.12 Molecular Weight V.S. Frequency shift

Ⅵ. CONCLUSION

In this paper, QCM measurement equipment is proposed. In the result, the short-term stability in liquid environment is below 0.01ppm (0.09Hz) per second. When antibody is attached to the crystal sensor, the sensor response shows long term stability of 0.05ppm over 1.5hrs (0.45Hz). Immune reaction measurement was inspected the propriety with using this system. It was confirmed that the detecting sensitivity was improved thought telecommunication technology applied QCM by these results.

Ⅶ. COOPERATION RELATIONSHIP

This is the cooperation research result of Nihon Dempa Kogyo Co.,Ltd. With AIST.

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