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Frequency Stability of a Crystal Resonator for Biosensors

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Abstract- This paper describes oscillation frequency stability of a crystal sensor for biosensors used for detection of dioxins, PCB, environmental-stressors, and marker proteins for infections. Present frequency stability is about 0.1 ppm in 9 MHz. Design technology for communication is applied to the crystal unit and oscillator circuit as a sensor. We prepared a crystal unit for filters in an oscillator circuit, and adopted a method that engenders oscillation frequency stability. That method demonstrates performance of 5 ppb at 9 MHz and 14 ppb at 155 MHz.

I. INTRODUCTION

Research into use of quartz crystal units for biosensors is progressing. An all-quartz crystal sensor with of a sub-picogram level mass sensitivity has been reported [1]. However, in general, sensitivity of biosensors which use the present quartz crystal unit is on the nanogram scale [2]. A quartz crystal sensor with higher mass sensitivity is desired by industry for biosensing and environmental monitoring. Generally, mass sensitivity of a quartz crystal sensor is determined by oscillation frequency stability of an oscillator circuit. As a rough estimate, this oscillation frequency stability is determined by the following:

(1) Q value of a quartz crystal sensor;
(2) Q value of an oscillator circuit;
(3) Frequency and temperature characteristics by quartz crystal sensor and oscillator circuit;
(4) Measurement environment temperature stability;
(5) Measurement system stability.

The colpitts circuit is applied to quartz crystal oscillator circuits for sensors. However, improvement of the Q value of an oscillator circuit and improvement of frequency temperature characteristics of an oscillator circuit of inclination are not studied. For this reason, present frequency stability is about 0.1 ppm level in 9 MHz; 0.1 ppm mass sensitivity is about 1 ng.

On the other hand users require that this sensitivity be enhanced to the picogram or femtogram level. Frequency stability is required for the 1 ppb level when realizing the quartz crystal oscillator circuit with mass sensitivity of a picogram. Users of communication equipment regard such frequency stability as a general specification. Such frequency stability may be attainable with a quartz crystal unit that uses design technology similar to that for an oscillator circuit used for communication devices.

From such a perspective, the quartz crystal unit for communication system, the oscillator circuit, and the sensor and oscillator circuit that use a quartz crystal unit using frequency measurement technology are all reexamined to raise mass sensitivity of a quartz crystal unit sensor from the nanogram level to the picogram level. This paper also considers frequency stability improvement. Frequency stability is 5 ppb at 9 MHz and 14 ppb at 155 MHz. Finally, results are described herein.

Results of this research from Nihon Dempa Kogyo Co., Ltd. were obtained in cooperation with research of the AIST.

II. MATERIALS AND METHODS

2.1 Design specifications of a quartz crystal unit

This chapter explains design specifications of a quartz crystal unit.

Chemical reagents perform chemical treatment of the crystal blank. Thereby, frequency stability of a quartz crystal sensor is worsened. A grade Aa quality synthetic quartz crystal blank has been used as the basic material to improve it [3]. On the other hand, a crystal blank of a flat board has also been used [4].

Frequency and temperature characteristics of a crystal unit are important design elements. The cut angle of a crystal blank determines frequency temperature characteristics of a crystal unit. Moreover, rotating frequency temperature characteristics of a crystal unit have been observed when a load is applied to the crystal blank surface [5]. Frequency and temperature characteristics of the crystal unit (as crystal sensor) are changed by character, thickness, etc. of an antibody function as a load on the crystal blank surface. For this reason, this change must be rectified. The crystal sensor cutting angle must be determined such that it may become zero mostly about inclination of frequency to temperature.

Fig. 1 shows the result of verifying and checking this phenomenon by experiment. Curves shown in Fig. 1 are the result of measuring frequency and temperature characteristics of the crystal unit in which the plate is formed. Near normal temperature and frequency inclination near zero are observed.

Next, thickness is adjusted to is about one micrometer in the plate on one side of a crystal blank with acrylic acid (it reacts easily with water). Then, frequency and temperature characteristics are measured. Fig. 1(b) shows this result.

As a result, if acrylic acid is formed, correct frequency and temperature characteristics will be obtained. Next, the lid is carried out for another crystal side to the silicone system epoxy using the crystal blank. Then, frequency temperature and characteristics are measured again. Fig. 1(c) shows this result. Then, correct adjustment of frequency temperature characteristics continues.

Finally, this resonator is put in into ultra-pure-water; then, frequency and temperature characteristics are measured while heating this water. Fig. 1(d) shows these results. Correct determination of frequency and temperature characteristics is advanced. Then, a crystal sensor is put into ultra-pure-water and frequency temperature and characteristics of this crystal sensor are measured.

The cutting angle of a crystal blank is accordingly chosen based on this result; consequently, it is important to reduce inclination of frequency and temperature characteristics near the measurement temperature to near-zero.

The crystal unit used as a sensor here is produced in this manner, by selecting the cutting angle accordingly and adjusting it. Inclination of frequency temperature and characteristics near normal temperature are smaller than 0.01 ppm/°C at +25°C +/-1°C.

The plate material comprises two layers: Cr and Au. The Au requires Cr to ensure adherence to a crystal blank. Thickness of Au and thickness of Cr are 100Å to about 1500Å. The Cr is influenced by an antibody; it twists for detection [6] [7].

Crystal sensors either use support systems or do not. The following method does not use a supporter.

(a) How to contact the container with the depth like melting pot to a direct crystal blank and to burden with the duty of a support.
(b) A support is not used, but a crystal blank is a method fixed with a conductive epoxy.

Fig. 2a shows the method of contacting a container with the depth like melting pot to a direct crystal blank, and burdening with the duty of a support is the method of fixing the whole end surface of a crystal blank. This has the function of suppressing elastic vibration of a crystal unit, changing frequency with time, and changing frequency temperature characteristics. Therefore, it should not be adopted if considered from the viewpoint of crystal unit frequency stability [8]. The method of fixing a crystal blank with a conductive epoxy, although a supporter is not used as in other methods, is used for cellular phones or the crystal units for automobile-related devices.

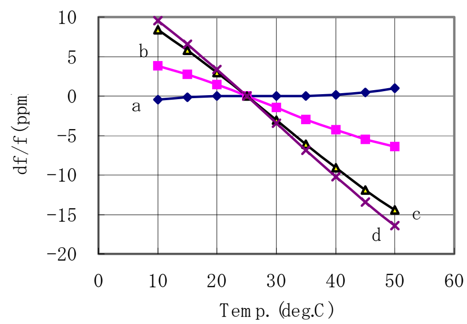

Fig. 1. Frequency and temperature characteristics of a 155 MHz crystal unit.

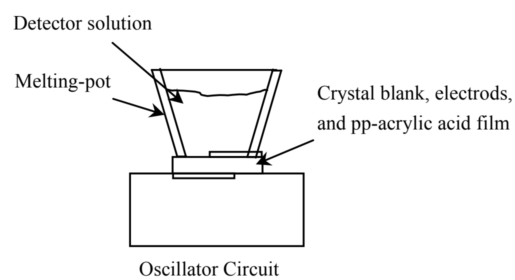

Fig. 2a. Composition section of a sensor by melting pot.

On the other hand, a method using a supporter in the structure of the crystal unit is shown in Fig. 2b. These composition and structure of the crystal unit are used currently, but this method is outdated as an object for communication apparatus. Devising a design support, a crystal oscillator in which the "method of using a support" or the "method of fixing a crystal blank with a conductive epoxy although a support is not used" offers realization of frequency stability of ppb/day. This method is already applied to a part of OCXO (Oven Controlled Crystal Oscillator) for GSM base stations. Table 1 shows design specifications.

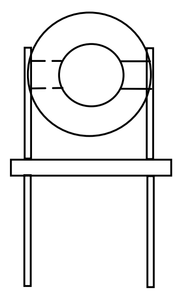

Fig. 2b. Structure of a quartz crystal unit by supporter.

Table 1
Design of quartz crystal units

Nominal Frequency (MHz)	9	30	50	155
Enclosure	HC-49/U	HC45/U		NX3838SA
Quality grade of synthetic quartz	IEC 60758 Grade Aa			
Finish	WA#3000	Polish		
Electrode Material	Cr, Au			

Design of plasma-polymerization of acrylic acid was based on the following conditions. Monomers of acrylic acid used in this experiment are all of analytical grade and purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); they are used without further purification. For plasma polymerization (pp-), pp-equipment is used (BP-1; Samco International Co., Kyoto, Japan). Deposition rates of pp-acrylic acid films are measured *in situ* with a QCM during polymerization. A QCM with gold electrodes on both surfaces is used (AT-cut of 9 MHz: 8 × 8 × 0.15 mm). The pp-films are deposited on one side of the QCM. Prior to plasma polymerization, the six QCMs are treated by plasma sputtering for 5 s under 100 W RF power and 100 Pa of He pressure. Two parameters control plasma polymerization conditions: monomer vapor pressure and glow discharge power. We used these polymerization conditions: a 100 Pa vapor pressure for each monomer; RF power of 100 W. Plasma polymerization of acrylic acid is performed for 5 to 300 s. We obtained an optimum film-state of polymers under these conditions [9].

II.3 Oscillator Circuit and Measurement System

This chapter describes the oscillator circuit composition and oscillation frequency measurement system used for experimentation.

A method to improve a sensor's frequency stability and mass sensitivity is as follows.

(1) The method of using jitter filter is one method for reducing the noise component within an oscillation signal.
(2) Effective Q of an oscillator circuit is increased. For example, this is the method of establishing a crystal unit for filters, and a tank circuit in an oscillator circuit. The method proposed here is the latter method [10].

A colpitts circuit was used for the crystal oscillator. The base grounding type was applied to this oscillator circuit. This oscillator circuit can realize a high impedance input. A coil or the crystal unit is used for the filter into the oscillation loop in this oscillator circuit. Oscillation output was obtained from the crystal sensor terminal. The waveform plastic surgery circuit, which makes a square wave, is adopted in the following stage; the interface with other attached equipments is secured.

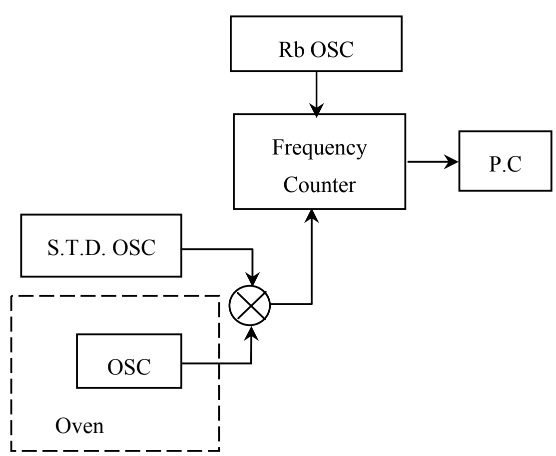

Fig. 3. Block diagram of measurement system.

In this measurement system, oscillation frequency read 0.1 ppb at 9 MHz: 0.0009 Hz, 30 MHz: 0.003 Hz, 50 MHz: 0.005 Hz, and 155 MHz: 0.01 Hz.

To attain frequency stability capable of allowing detection 1 ppb, measurement error must be prevented by a gating-error counter. Then, to prevent measurement error by the gating-error counter, a heterodyne detecting method, which is currently utilized for communication equipment, is used. Fig. 3 shows this measurement block diagram. This block diagram is the general composition as an object for communication equipment. This measurement system is connected and controlled by GPIB. This system is set in room temperature +25°C + / -1°C.

III. RESULTS AND DISCUSSION

This section presents frequency stability measurement results. Measurement is started after preparation. Results are the following.

(1) Acrylic acid is formed in the crystal side; it then reacts with ultra-pure-water. Resonance frequency of a crystal oscillator changes.
(2) Then, reaction stopped and change of this resonance frequency was stabilized.
(3) Fig. 4 expresses the oscillation frequency change for 4 min after measurement began.
(4) After starting measurement, oscillation frequency changed to the minus side in about 0.9 min.
(5) It changed to -110 ppm after about 1.5 min.
(6) Oscillation frequency became stable.

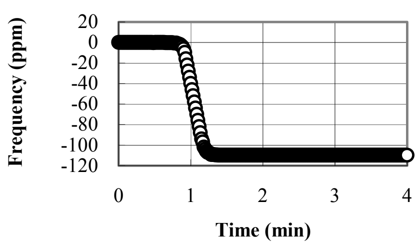

Fig. 4. Typical measurement result.

Fig. 4 shows a distribution of this oscillation frequency stability measurement result of 9 MHz. Crystal sensor frequency are 9 MHz, 30 MHz, 50 MHz, and 155 MHz. After starting measurement of oscillation frequency, the oscillation frequency of 60 min after was set as the standard. The oscillation frequency for the next 60 min was recorded from this standard. Fig. 5 shows results of arranging data for these 60 min. Statistical values of measurement results are shown in Fig. 5.

(1) 9 MHz: Avg. 1 ppb; std. 1.6 ppb
(2) 30 MHz: Avg. 2 ppb; std. 2.2 ppb
(3) 50 MHz: Avg. 2 ppb; std. 2.3 ppb
(4) 155 MHz: Avg. 3 ppb; std. 4.5 ppb

Generally, the range of the concerned sensor is *ca.* 1-2 h; it measures the amplitude of oscillation frequency change. Using these results, the Sauerbrey formula [11] computes and determines the amount of reactions as a sensor. Frequency middle stability is important for the sensor oscillation signal. Consequently, we infer that time-frequency which is stable is almost uniform. Therefore, although it is influenced by plate area, middle frequency stability required as an object for sensors is about 5 ppb at 9 MHz, about 6.6 ppb at 30 MHz, about 7.7 ppb at 50 MHz, and about 14 ppb at 155 MHz. This result is achieved by calculating standard deviation × 3. It is expected that picogram level sensitivity can be obtained from these results.

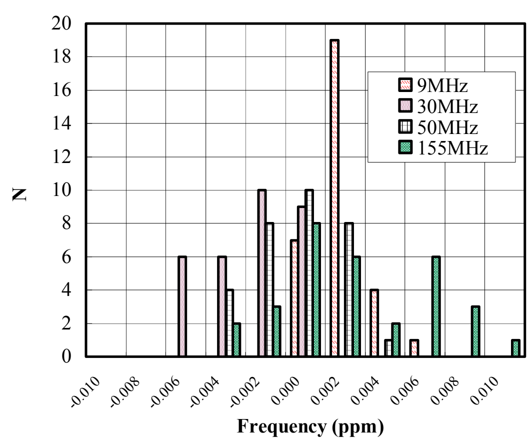

Fig. 5. Frequency stability of quartz crystals.

IV. CONCLUSION

We intended to produce a picogram-scale mass sensitivity biosensor using a nanogram crystal unit. We used components applied to communication equipment, design technology of a crystal unit, an oscillation circuit, and frequency measurement technology. Then, a design of the whole biosensor, design technology of an oscillator circuit, and frequency measurement technology were reviewed. Frequency stability improvement was achieved. Frequency stability is described as 5 ppb at 9 MHz and 14 ppb at 155 MHz.

Future subjects include improvement of the crystal unit structure used as the main part of the sensor, and improvement of the effective Q value of the oscillation circuit.

References

[1] H. Sota, Y. Okahata, *et al.*, “A versatile planar QCM-based sensor design for nonlabeling biomolecule detection,” *Anal. Chem,* 74 (15), 2002, pp. 3592-3598.

[2] E. Uttenthaler, *et al.*, “Ultra sensitive quartz crystal microbalance sensors for detection of M13-Phages in liquids,” *Biosensors & Bioelectron.*, 16, 2001, pp. 735-743.

[3] IEC 60758, “Synthetic quartz crystal - Specification and quid the use”.

[4] M. Koyama, *et al.*, “Fundamental mode 155 MHz with flat blank in the crystal using VCXO,” *Proc. 1999 Joint Meeting EFTF - IEEE IFCS*, 1999, pp. 338-342.

[5] M. Nakazawa, M. Suzuki, “An analysys of frequency temperature characteristics *vs.* film thickness of electrodes for AT-cut resonators,” *Proc. 1997 IEEE IFCS*, 1997, pp. 588-595.

[6] M. Koyama, Y. Watanabe, H. Sekimoto, and Y. Oomura, “A novel technique for measuring drive level dependence of quartz units,” *Proc. 9th EFTF*, 1995, pp. 16-19.

[7] M. Koyama, Y. Watanabe, H. Sekimoto, and Y. Oomura, “An experimental study of long term aging of quartz oscillators,” *Proc. 1995 IFCS*, 1995, pp. 620-622.

[8] J. R. Vig, “Quartz crystal resonators and oscillators for frequency control and timing applications,” *Proc. 1997 IFCS Tutorial*, pp. 3-38.

[9] S. Kurosawa, T. Hirokawa, *et al.*, “Detection of deposition rate of plasma-polymerized films by quartz crystal microbalance,” *Thin Solid Films*, 374 (2), 2000, pp. 262-267.

[10] T. Uchida, M. Koyama, *et al*., “A low phase-noise oscillator design for high stability OCXO’s,” *Proc. 1996 IFCS*, 1996, pp. 747-751.

[11] G. Sauerbrey, “Verwendung von schwingquarzen zur wägung dünner schichten und mikrowägung,” *Z. Physik*, 155, 1959, pp. 206-212.