# Measurement of QCM Sensor Coated With Biological Membrane in Gas Phase

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# Contents

# Abstract

Although various sensor elements for sensing odorants have been proposed up to now, sensor elements with sufficient sensitivity and selectivity still have not been realized. Therefore, there is a possibility of realizing an olfactory sensor with high performance by odorant binding protein (OBP) or olfactory receptors (OR) utilizing the function of living organisms. However, those sensors have been limited to sensing in the liquid phase, and sensing in the gas phase can not be performed. The purpose of this research is to develop odorant sensors with biological materials and realize highly sensitive and highly selective sensors in the gas phase. We take 3 means to realize the purpose. First, we use the odorant binding protein and the cell expressing olfactory receptor. Second, we choose the quartz crystal microbalance as the transducer of signal. Third, we establish the automatic measurement system which is able to measure at high humidity environment. After the response experiments of QCM sensors coated with OBP or cell expressing OR, we had 3 achievements in this study. First, we improved the measurement system. We embedded the solenoid valve controlling board into the measurement system. The system can automatically control the sequence to change the relative concentration of sample gas and measure the response in gas phase. Second, an OBP was found to have potential for odor sensing. QCM sensors coated with OBPs were more sensitive than the QCM sensors coated with typical chemical sensing film such as Siponate DS-10 with the same amount of coating. A.gam OBP4 has better sensitivity than A.gam OBP1. Pig OBP1 is considered to have the selectivity between benzaldehyde and amyl acetate from the comparison of the frequency shift. High humidity may enhance the sensitivity of OBP to the odorant sample. Third, in the experiment of QCM sensors coated with the cell expressing OR, it was found that Or56a had the selectivity with geosmin and Or13a had the selectivity with 1-octen-3-ol even in the gas phase. High humidity may also enhance the sensitivity of OR to the odorant sample. The life time of QCM sensors coated with Or56a and Or13a could be longer than 10 days. QCM sensors coated with Or56a is considered to have longer duration time than that coated with Or13a. We are looking forward to conducting further experiments on broadening the range of samples or changing the storage method of QCM sensors to find more properties of QCM sensors coated with biological membrane.

# Chapter 1 Introduction

## 1.1 Research background

An odor sensing system is required in many fields such as food, beverage, cosmetic, environmental system, etc. In our laboratory, we have been studying odor identification and reproduction using QCM odor sensor. We have for a long time been involved in research and development of odor sensing and odor recorder systems. [1][2]

In order to detect the odorant, various sensors such as quartz crystal microbalance sensor, surface acoustic wave sensor, a metal oxide gas sensor, a conductive polymer sensor, electrochemical sensor, MOS (Metal Oxide Semiconductor) gas sensor, an optical sensor have been proposed. However, it is indispensable to improve the sensor performance.

The basic concept of the odorant sensor is to recognize the output pattern of multiple sensors by pattern recognition. This idea was proposed by K.C.Persaud. Since he proposed the method using pattern recognition, many researchers mainly in Europe entered this field. Though more than 30 years have passed since the proposal, it has not reached a stage of being popular. This is because that a sensor having sufficient sensitivity and selectivity as described above has not been realized. Recently, in Europe, there is a proposal of a large-scale sensor array composed of more than 10,000 elements. However, there are many sensors with similar characteristics, and actually the number of sensors with different characteristics is not large even if the number of elements is 10,000 or more.

On the other hand, sensors utilizing olfactory receptors of living organisms have been studied. It is expected that highly sensitive and highly selective sensors can be realized using olfactory receptors since the living organism has excellent capability. By using olfactory receptor cells as sensing materials, sensors using various transducers have been considered. At the beginning, the electrode was inserted into the cell and potential measurement was performed. However, a non-invasive method using fluorescence was developed. There was an important problem of this measurement that we were only able to detect the odorant in the liquid phase although the odorant should be detected in the gas phase.

1.2 Related research about odor biosensor and QCM sensor

Prof. Persaud of Univ. Manchester has spent much time on studying about the odorant binding protein (OBP). The OBP used in this study is provided by his laboratory.

OBP is discovered in vertebrates and insects almost simultaneously. It was hypothesized that they could be responsible for recognizing olfactory stimuli acting as carriers of scavengers for hydrophobic molecules of odorants, but their exact role in olfaction is still uncertain. It is largely agreed that OBPs function as solubilizers for odorants. [3]

Prof. Persaud constructed an OBP biosensor with OBP and QCM sensor. Because the amino-acid sequences of many OBPs are available, he was able to create different kind of OBP. Proteins were immobilized on the gold surfaces of the quartz crystals using methodology of covalent immobilization – Self assembled monolayer (SAM).

With the odor biosensor he fabricated, he did some sensor response experiments.

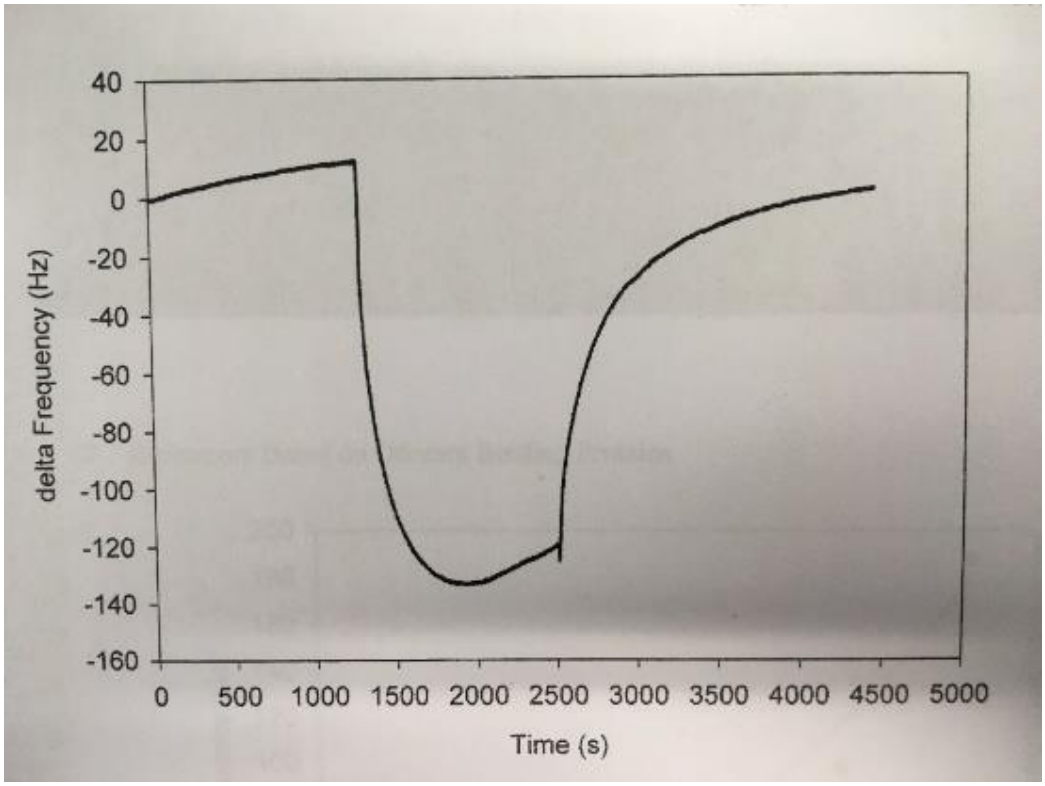


Fig. 1-1 Raw response signal from a QCM [3]

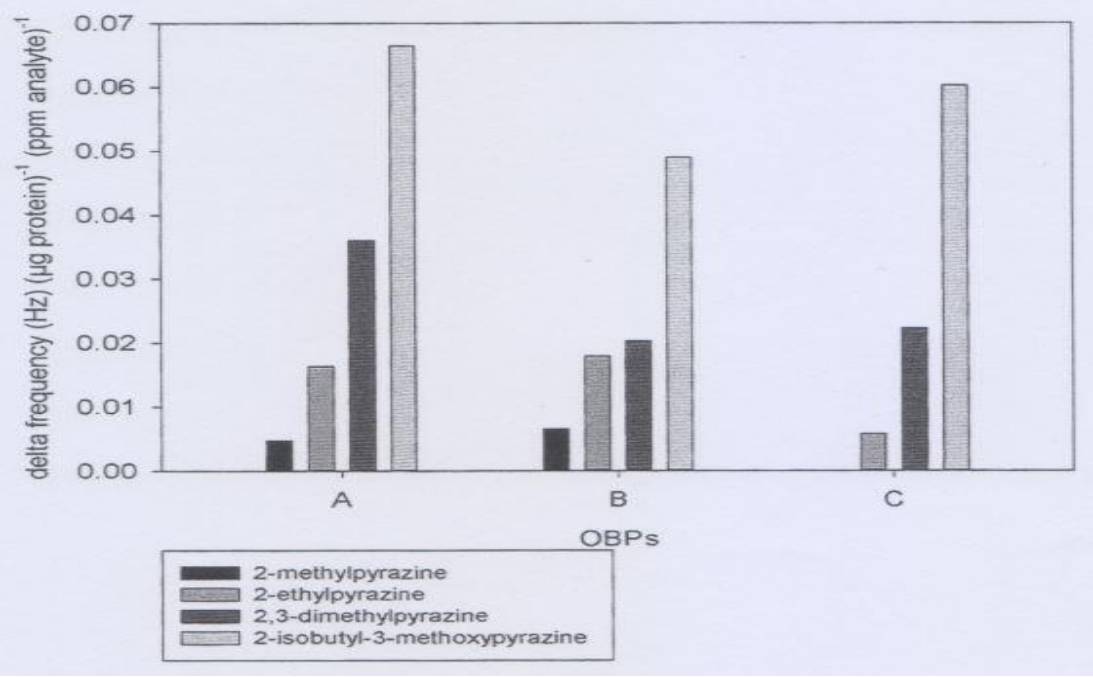


Fig. 1-2 Normalised responses of 3 kinds of OBP immolibized on QCMs to different pyrazines [3]

Fig. 1-1 shows the raw response signal from a QCM with immobilized odorant binding proteins from the Polistes dominulus to 350ppm of 2-isobutyl-3-methoxypyrazine in air. The sensor showed good reversibility as well as stability over time.

He has also investigated the selectivity of proteins from different sources to a range of different pyrazines. Fig. 1-2 shows the normalised responses of 3 kinds of OBP immobilized on QCMs to different pyrazines.

From his study about OBP, we confirm that using a QCM platform as a transduction element, it is possible to detect and measure quantitatively concentrations of volatile analytes at ppm concentrations in air.

By changing the coating material of the QCM sensors, the application will also change.

ASIC and System State Key Lab from Fudan University has coated Ag+-ZSM-5 zeolite film on QCM sensor and realized a highly sensitive QCM sensor for medical diagnosis. [4] Ag+-ZSM-5 zeolite is a new zeolite material for diagnosis of diabetes. It has nanometer cavities, the diameter of which approximates uniformly 5.0A, which is very close to the molecular size of acetone (about 4.4A). When the zeolite is spin-coated on the QCM, the

nanometer cavities trap the molecules smaller than its diameter and cause a mass change of the QCM.

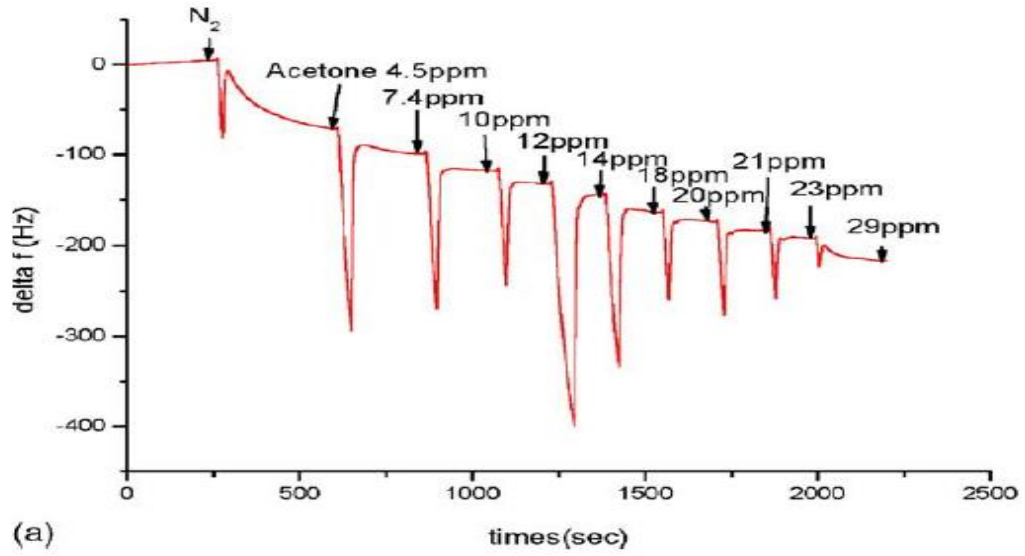


Fig. 1-3 The response of the QCM to different concentrations of acetone vapor in nitrogen [4]

Fig. 1-3 shows the sensor responses to the acetone vapour in nitrogen at different concentrations. The sensor response becomes larger with the increasing concentration of acetone.

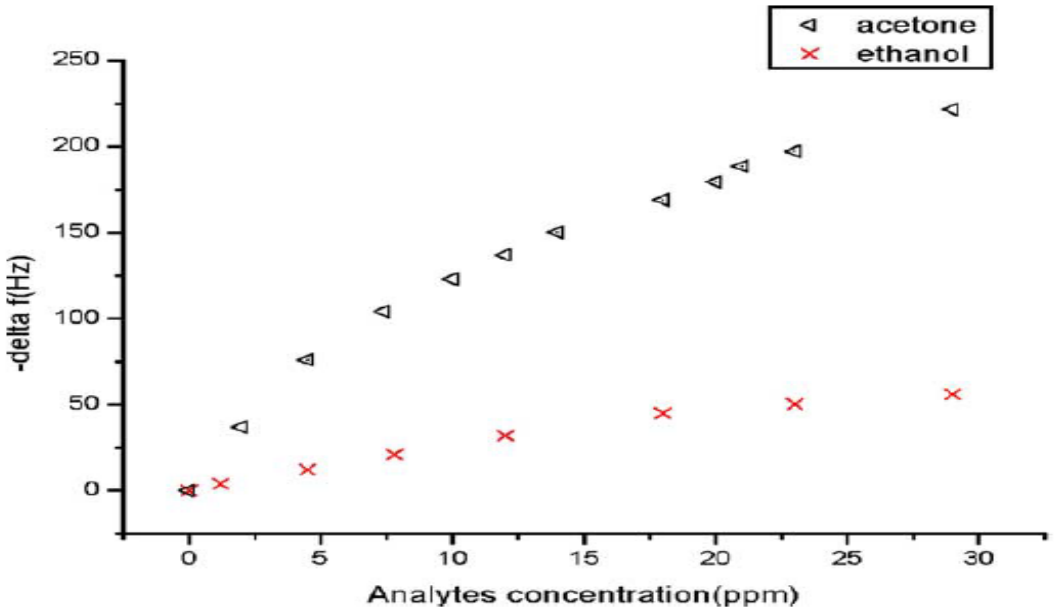


Fig. 1-4 The selectivity test of comparing the response to acetone with that to ethanol [4]

Fig. 1-4 shows that the sensor performs an insensitive characteristic to ethanol compared with acetone. This kind of QCM sensor has a special sensitivity and selectivity to acetone vapor.

1.3 Previous research about QCM sensors applied to odorant sensing

Mr. Wyzszynski in our lab has spent much time on the study of QCM odor sensors. He has studied about the sensing film of QCM odor sensors. [5]

He reported application of the PEGylated lipids (PEG lipopolymers) containing disulphide as supports for sensing films for quartz crystal microbalance (QCM) odor sensors. In this study, the materials are binding covalently to the surface of gold QCM sensor electrode creating self-assembled cushion. Additional amounts of lipid or lipid-derived materials can be physisorbed on that chemisorbed coating.

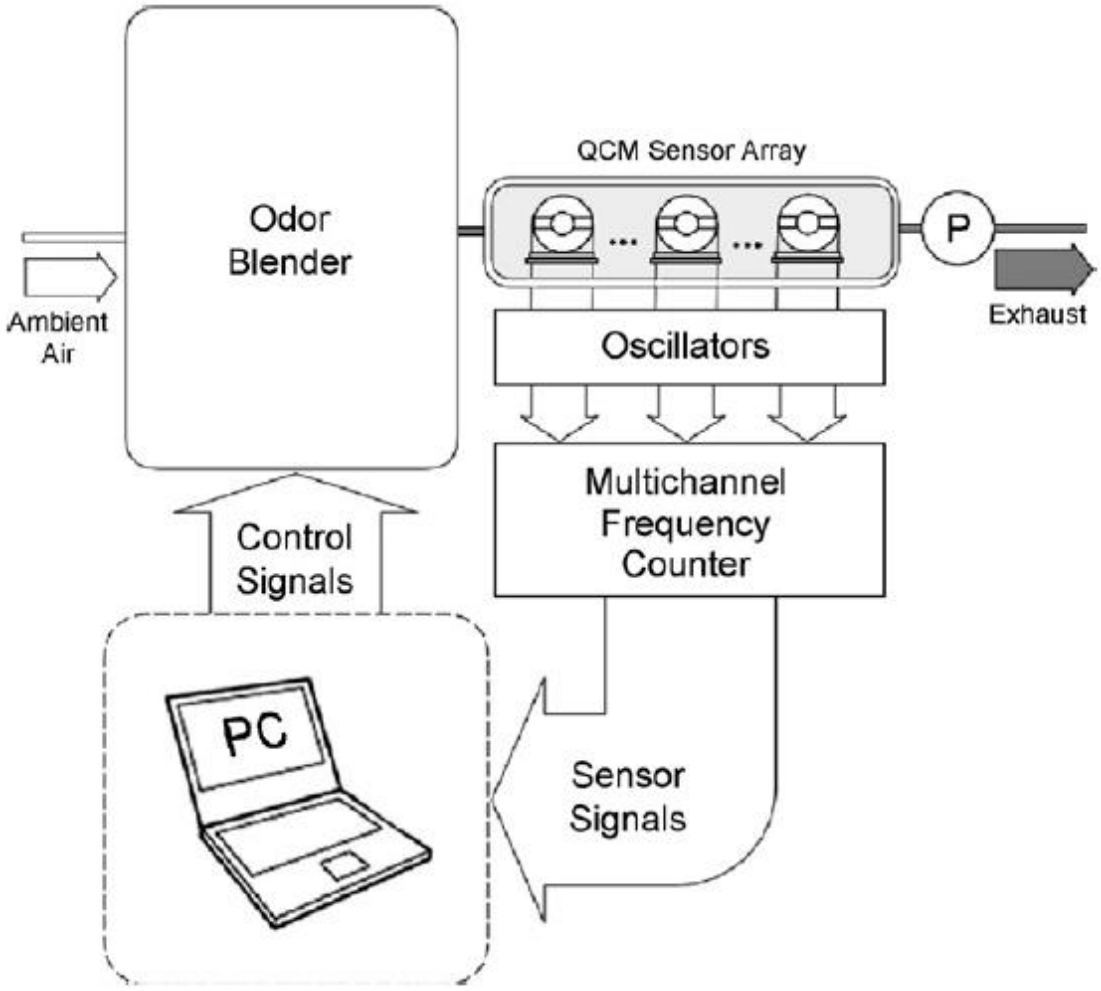


Fig. 1-5 Schematic of representation of the measurement system [5]

Fig. 1-5 shows the schematic of representation of the measurement system in this study. Dynamic headspace of odor samples was carried through the system into a sensor cell containing an array of QCM sensors. Ambient air, filtered with activated carbon, was used as a carrier gas. The sensor response acquisition and solenoid valve control were performed using a PC. The QCM sensors were installed in the measurement system for at least 12 h prior to the exposure experiments. The odorant samples were conditioned in the system for at least 30 min prior to the measurement. Each sensor was exposed to each odorant vapor three times.

The sensing materials studied here were three lipopolymers: PEG 1000 (LD1K), PEG3000 (LD3K),and PEG 5000 (LD5K), PDP and LD2K-PDP. The odorant samples were alcohols, acids, esters and aldehydes. The chemisorbed lipopolymers were used in the present paper as supports for the lipid and lipopolymer odor sensing materials on QCMs. He found that application of the supports influenced greatly the sensing properties of the resulting sensors. It was found that the sensors with support were more sensitive than their non-supported counterparts. The sensors were capable of discriminating 10 different odor samples.

Also he implemented the experiment of QCM sensors with lipopolymers and olfactory receptor-expressing cells for odor sensing in liquid phase. [6] Since the olfactory receptors (ORs) are known to bind quite specifically with molecules of a designated odorant (target odorant) such interaction should be detectable by using a mass-sensitive device. In this study we evaluate viability of this strategy by using the cultured cells with geosmin-specific ORs (Or56a). He used 9MHz QCMs with polished surface and gold electordes deposited on both side of the crystal.

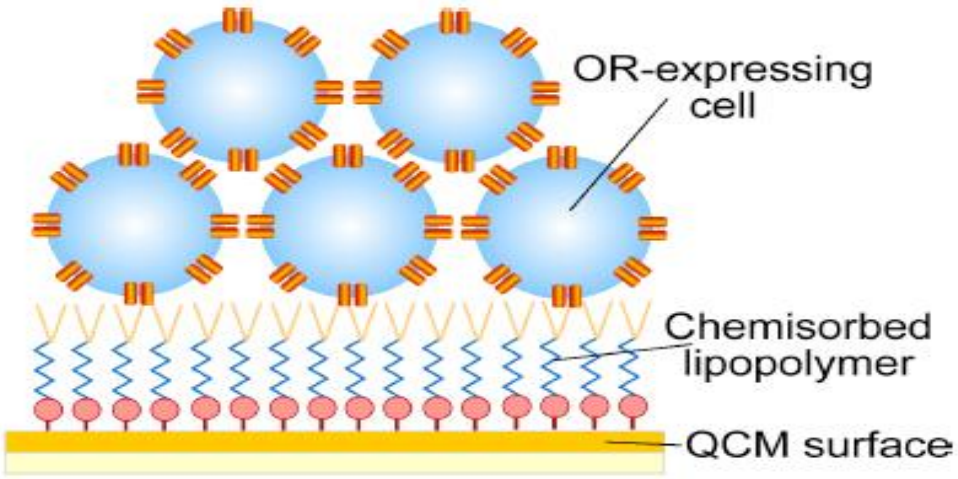


Fig. 1-6 Ideational representation of the fabricated sensing structure [6]

Fig. 1-6 shows the ideational representation of the fabricated sensing structure. After the fabrication of the sensing film, sensors were exposed to the solution of 4 odorant samples (Butyl acetate, n-butanol, Isobutyric acid, Geosmin) and DMSO (odorant solvent).

Responses to all 5 tested chemicals are summarized in form of a bar graph in Fig. 1-7.

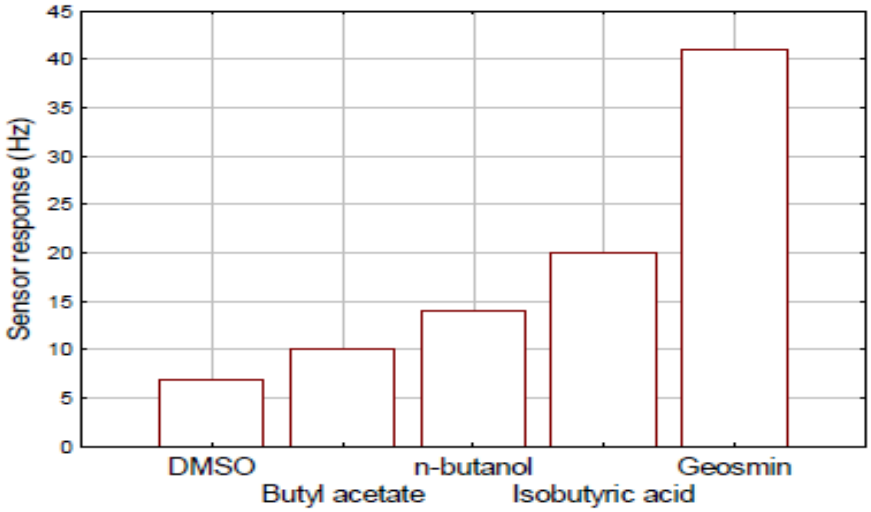


Fig. 1-7 Responses of the sensor to DMSO and 4 odorant samples at 360mM [6]

As can be clearly seen, the fabricated sensor exhibited quite high specificity toward the target odorant. Moreover, its application to an odor recorder might be challenging.

## 1.4 Research purpose of this study

In our lab, previous research about the cell expressing olfactory receptor were performed. From their research, we confirmed that cell expressing olfactory cell have the property of highly selective odorant molecule in the liquid phase. Previous research about the QCM odor sensor was also performed in our lab. The QCM sensors coated with different sensing films such as lipopolymer, cell expressing olfactory receptor were tested in liquid phase. From that research, we confirmed that QCM sensor has the ability of acting as a good signal transducer in the liquid phase. Moreover, we have opportunity to use odor binding protein. Although odorant binding protein has the main role of carrying odorant molecule to the olfactory receptor, the protein itself still has the ability of recognizing odorant molecule and has the potential of sensing odorant. We expect that it can still has high sensitivity and high selectivity in the gas phase by supplying high humidity environment to QCM sensor coated with biological membrane.

In this study, the purpose of this research is to develop odorant sensors with biological materials and realize highly sensitive and highly selective sensors in the gas phase.

We take 3 means to realize the purpose.

First, we use the odorant binding protein and the cell expressing olfactory receptor.

Second, we choose the quartz crystal microbalance as the transducer of signal.

Third, we establish the automatic measurement system which is able to measure at high humidity environment.

# Chapter 2 Sensor element developed in this study

## 2.1 QCM sensor

Quartz crystal microbalance is used as the transducer of single in this study. It has a structure that a metal thin film is attached to both sides of quartz crystal which is cutting into an ultrathin plate shape. It shows the property of oscillating at a certain frequency when an alternating electric field is applied. When substances on the order of nanogram are adsorbed on the metal thin film, the resonance frequency decreases in proportion to the mass of the substance. So it can be used as a microbalance.

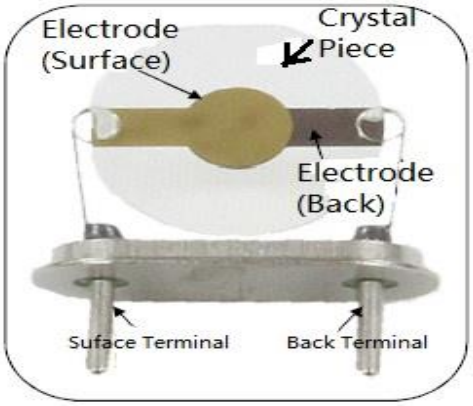


Fig. 2-1 Structure of QCM

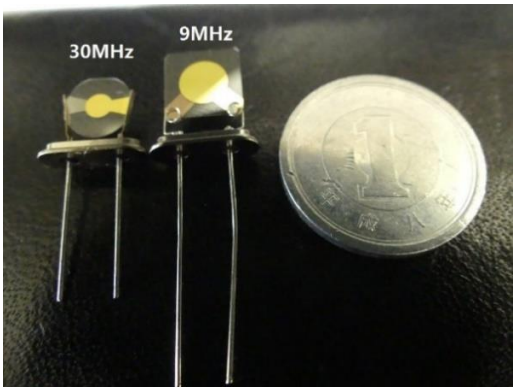


Fig. 2-2 9 and 30MHz AT-CUT QCMs used in this study

Fig. 2-1 shows the structure of a typical QCM sensor. It is composed of 2 electrodes, 1 quartz crystal piece and 2 terminals. Fig. 2-2 shows the exact 9 and 30MHz QCM sensors used in this study.

## 2.2 Odorant binding protein

The odorant binding protein is a protein to carry an odorant to an olfactory receptor in the mucus layer [6]. The numerous OBPs were discovered and it was found that they have the selectivity for odorant subclasses. Thus, there is possibility that they work as odor sensors. The OBPs are useful since they work even in the gas phase for a long time.

Fig. 2-3 shows the function of OBP. There is a water-based fluid layer

between the surface of antennae sensors and the olfactory nerve beneath. Chemical compounds, however, are almost invariably insoluble in water and therefore unable to cross the thin aqueous layer by themselves. The OBPs encapsulate the scent chemicals in a water-soluble coating giving them a free access through the layer. Negatively charged receptors on the surface of the olfactory nerves cause a structural change in the surface coating which causes the encapsulated scent chemical to be ejected onto the nerve receptor.

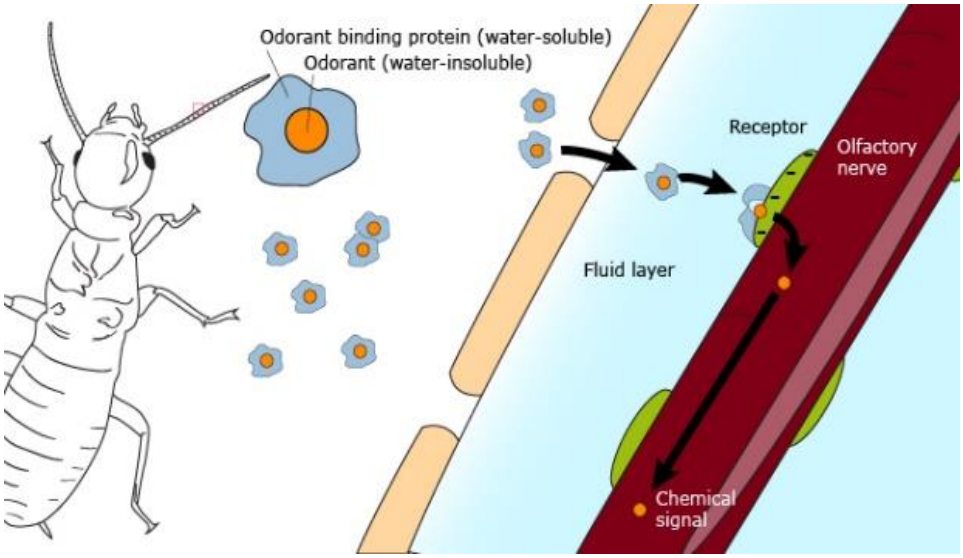


Fig. 2-3 Function of OBP [7]

To immobilize the OBP on the surface of QCM sensor, we use the method of self-assembled monolayer (SAM).

Fig. 2-4 shows the representation of a self-assembled monolayer structure. Self-assembled monolayers of organic molecules are molecular assemblies formed spontaneously on surfaces by adsorption and are organized into more or less large ordered domains. SAMs are created by the chemisorption of "head groups" onto a substrate from either the vapor or liquid phase followed by a slow organization of "tail groups". In this experiment, OBPs are immobilized on the surface of QCM sensors via SAM.

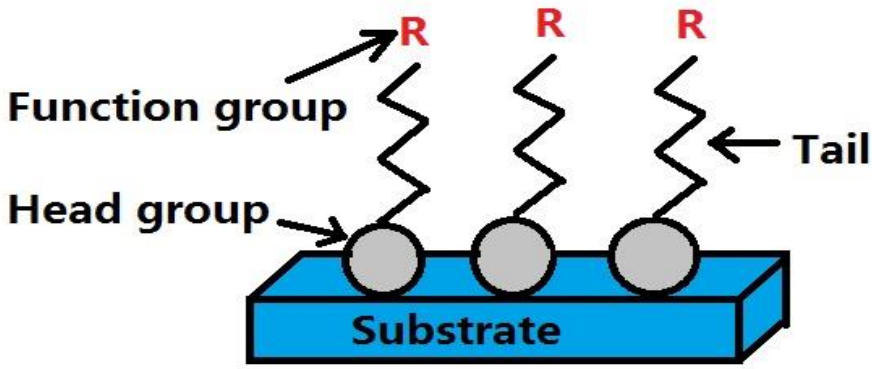


Fig. 2-4 Representation of a SAM structure

In this study, 3 kinds of OBPs provided by Prof. Persaud were used. They were called A.gam OBP4, A.gam OBP1 and Pig OBP1. A.gam OBP1 and A.gam OBP4 were taken from mosquito and Pig OBP1 was taken from pig.

Prof. Persaud's immobilization procedure is shown as follows:

1. Thioctic acid (TA)(100mM in absolute ethanol) is dropped on the gold surface of the crystal, repeatedly every 20 min, for at least 2 hours. The same procedure is used for the gold electrode on other side of the quartz crystal. The SAM procedure is carried out in a controlled environment under nitrogen.
2. The quartz crystal is then rinsed with an excess of absolute ethanol and is left to dry at air. In order to activate the carboxylic acid groups of the SAM, 20ul of a solution consisting of a mixture of ethyl(dimethylaminopropyl)carbodiimide (30mM) and N-hydroxysuccinimide (60mM) is placed on the gold surface for 2 hours.
3. The solution is then rinsed with distilled water and is dried at air.
4. The immobilization of proteins on the activated SAM layer is carried out by pipetting the OBP solution onto the gold surface and leaving it for 1 hour before gently rinsing with distilled water and drying in air. The amount of protein deposited on to the gold surface corresponds to about 10 ug of OBP. [3]

In this study, the procedure was simplized. It was performed by Mr. O.Dani. The procedure is shown as follows:

First, we drop the lipoic acid (LA) on the gold surface of the crystal. The sulphur atoms of the LA forms a strong bond with the gold, while the other

end of the molecule is free to bind to the proteins. Then, we rinsed the quartz crystal with ethanol and let it to dry at air. After that, a mixture of ethyl (dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide was placed on the gold surface for 2 hours. These procedures for the SAM activation were applied to both sides of the gold surfaces. The immobilization of proteins on the activated SAM layer was carried out by pipetting the OBP solution onto the gold surface and leaving it for 1 hour before gently rinsing with distilled water and drying in air.

2.3 Olfactory receptor

Olfactory receptors (ORs) are expressed in the cell membranes of olfactory receptor neurons and are responsible for the detection of odorants. In vertebrates, the olfactory receptors are located in both the cilia and synapses of the olfactory sensory neurons and in the epithelium of the human airway. In insects, olfactory receptors are located on the antennae and other chemosensory organs.

The structure of cells expressing olfactory receptor is shown in Fig. 2-5. [8] Drosophila melanogaster's olfactory receptor (OR), olfactory receptor co-protein (Orco), and calcium-sensitive fluorescent protein are expressed in Sf21 cell derived from Spodoptera frugiperda. The odor response characteristics of the olfactory receptor of Drosophila melanogaster have been clarified, and the fabrication of sensor cells based on it has already been reported. In Fig. 2-5, it shows the principle that sensor cell detects fragrance substances and increase fluorescence intensity. Both OR and Orco are found on the cell membrane, and the two proteins form the ion channel. When a fragrance substance is accepted by OR, the ion channel opens and inflow of $Ca^{2+}$ into the cell occurs. The amount of inflow is dependent on the number of opened ion channels the same as the amount of aroma substances. On the other hand, GCaMP6s exists inside the cell, and fluorescence intensity increases by binding with $Ca^{2+}$.

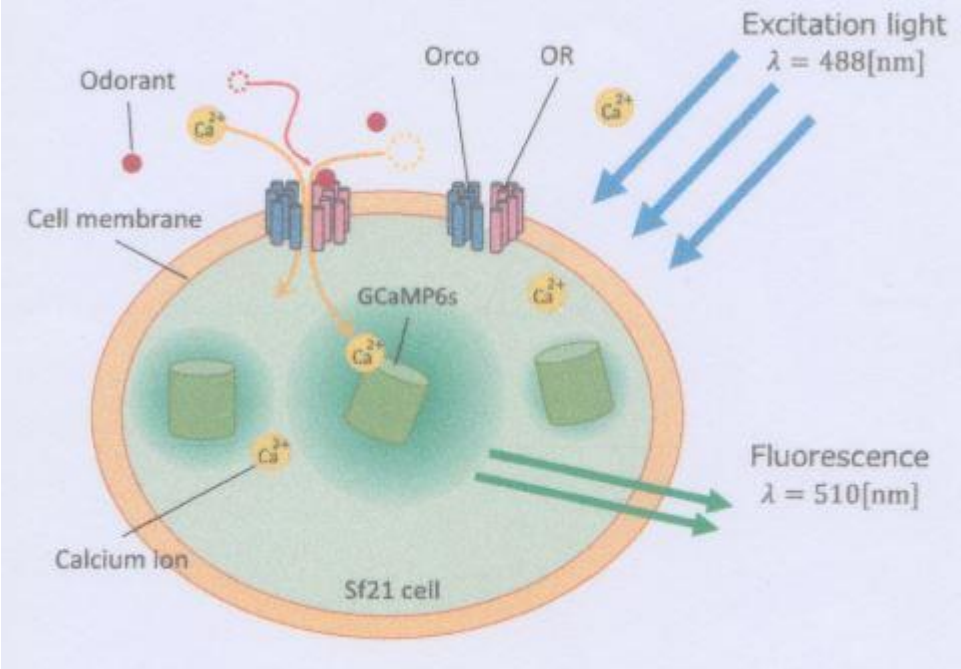


Fig. 2-5 Structure of the cells expressing olfactory receptor [8]

The sensor structure is extremely simple when QCM sensor is coated with cells expressing OR. We let the cells with ORs be absorbed on the surfaces of a QCM sensor as is shown in Figure 2-6. When an odorant substance is captured by an olfactory receptor, the oscillation frequency decreases due to the mass load effect. However, we need to keep the humidity of the carrier gas constantly at high level. By maintaining high humidity, it becomes possible to supply moisture to the cell and it is considered that the stereochemical structure of the olfactory receptor protein can be maintained. Then, a sensor using protein can work even in the gas phase.

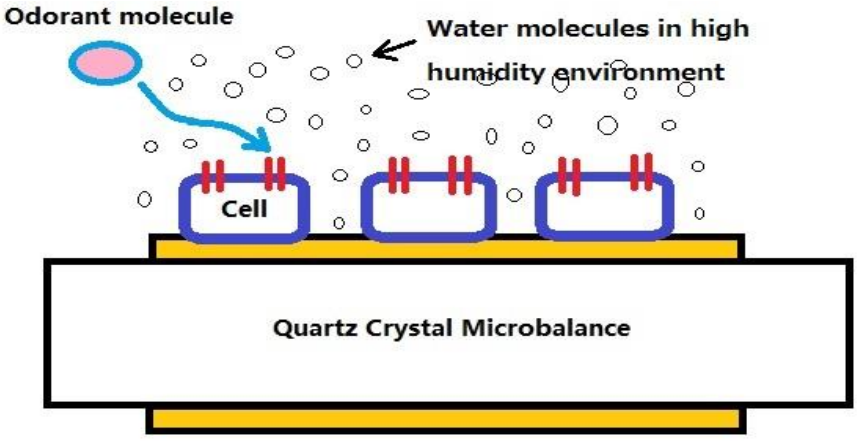


Fig. 2-6 Method of gas phase sensing using QCM

To coat a QCM with the cell expressing OR, first I get the cells from Mr. Sukekawa. The initial state of the cells is that it is cultivated in the box. We threw away the cultivating solution in the box. Then we used the cell scraper to let the cells be not adhered to the inner wall of the box. After that, we injected some ringer's solution no more than 10ml into the box and mixed it. Finally, we used the pipet to drop the cells mixed in 5-10ul ringer's solution on both surfaces of the QCM. We waited for about 30 minutes for the evaporation of the solution. And we measured the resonance frequency before and after coating by the network analyzer.

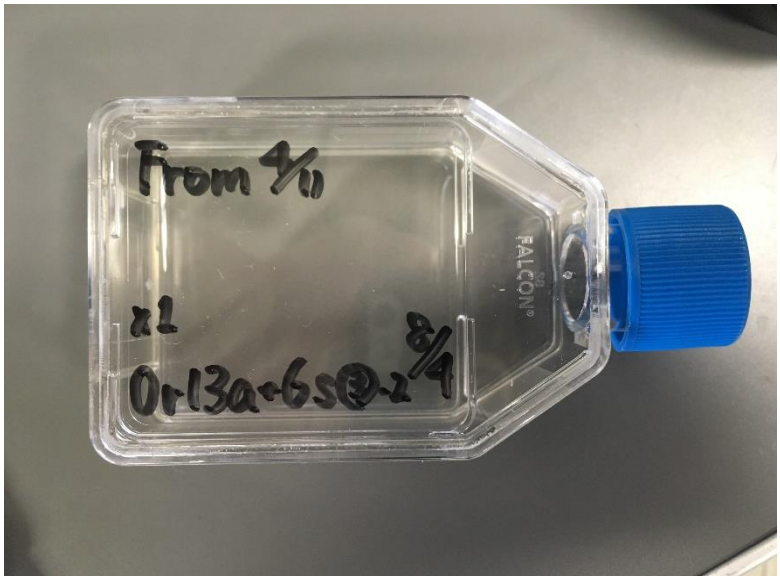


Fig. 2-7 Cells contained in the box

2.4 Summary

In this chapter, we introduced the sensor element in this study including the signal transducer – QCM and the coating material – OBP and OR.

# Chapter 3 Measurement system

## 3.1 Schematic of measurement system

The measurement system is demonstrated in Figure 3-1. It consists of mass flow controller (MFC), odorant blending system, sensing chamber with QCMs, a multi-channel frequency counter and computer for data acquisition and analysis.

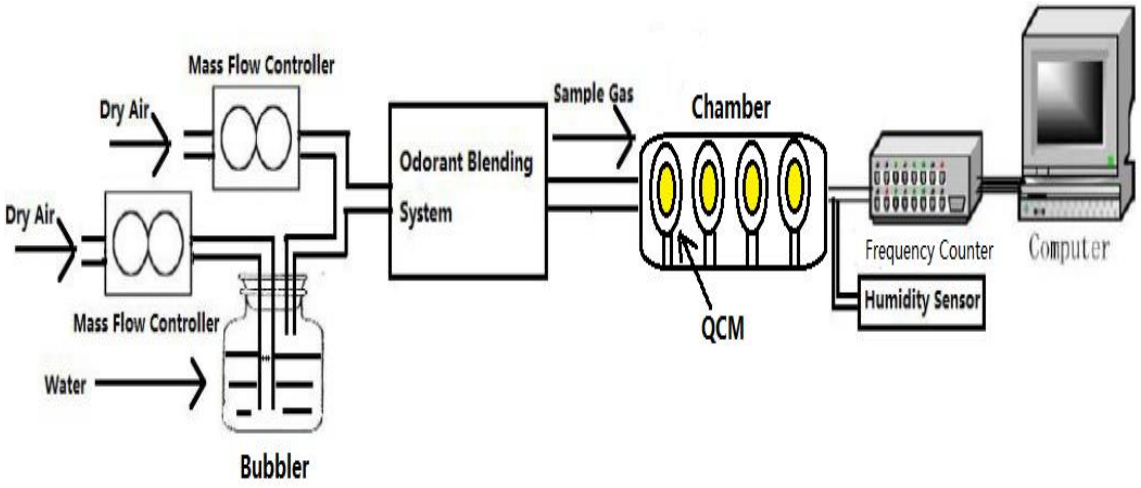


Fig.3-1 Schematic of Measurement System

Fig. 3-2 Bubbler used in this study

The bubbler contained with water shown in Fig. 3-2 is set for increasing the humidity of the sample gas.

Mass flow controllers were used to control the flow rate of the air and sample gas since the QCM is sensitive to the flow rate variation. The model number is SEC-400MK3 from HORIBA STEC Inc. The maximum flow rate is set at 500SCCM for the dry air and 200SCCM for the humidified air.

Odorant blending system is composed of 16 solenoid valves. It can change the relative concentration and the mixture composition if blended odor is used. [10] Fig. 3-3 shows the schematic of the odorant blending system.

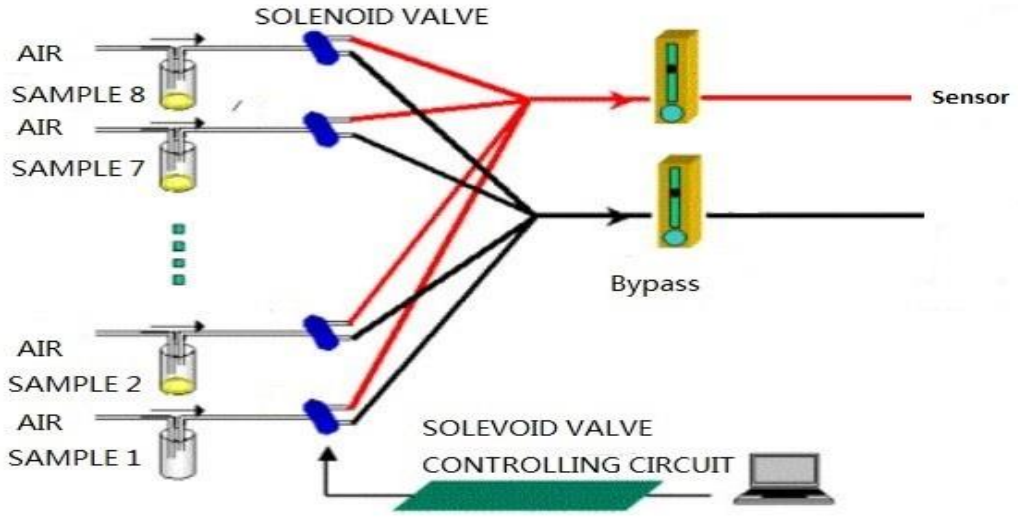


Fig. 3-3 Schematic of odor blending system

Up to 8 samples can be contained in this system. A pair of solenoid valves is responsible for 1 sample. Solenoid valve is like a switch which controls the flow path of air. Either of 2 solenoid valves is on to let the air or air mixed with odor sample go to the sensor chamber. The bypass way is set for maintaining the concentration of the headspace gas in the bottle and let it not be changed even the path is switched. We use the solenoid valve controlling circuit to control the duration time of solenoid valves. By setting the duration time of the on state, we can control the relative concentration of the sample gas. The resolution of the duration time we can set in software is 1ms. But because the signal is sent by computer using Matlab, it will has some delay.

Moreover, the solenoid valve itself needs a few tens of ms of ON time. The signal frequency is 1Hz.

The sensor chamber in this system can contain up to 8 QCM sensors as shown in Fig. 3-4. It enables us to measure 8 sensors at the same time.

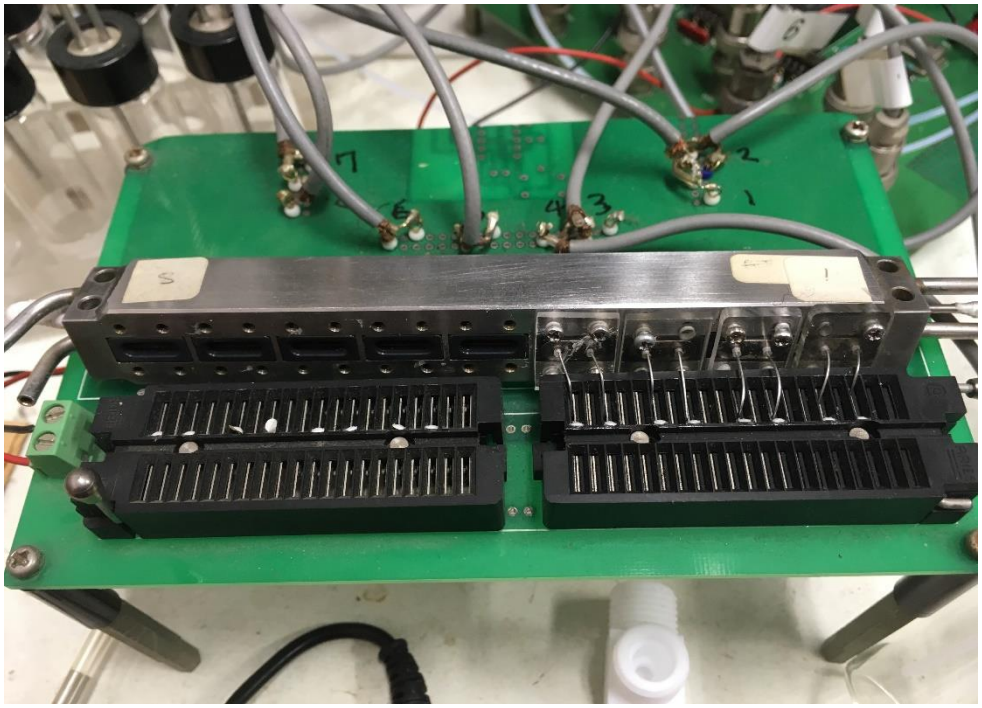

Fig. 3-4 Sensor chamber used in this study

Fig. 3-4 shows the sensor chamber made of stainless. It has nine slots for us to put the QCM sensor in. The terminals of the QCM can be put in the sockets of the green circuit board under the chamber. The circuit board is connected to the frequency counter.

8-channel frequency counter with 1Hz resolution is used to measure the oscillation frequencies of QCM sensors and we used Matlab (Mathwork Inc.) to collect the data and to analyze them. The circuit board of the frequency counter is shown in Fig. 3-5. The cables in the bottom part of the board are connected to the circuit board under the sensor chamber. In the upper part, the cables are connected to the computer by serial communication. The number of the bit of every channel is 14bits.

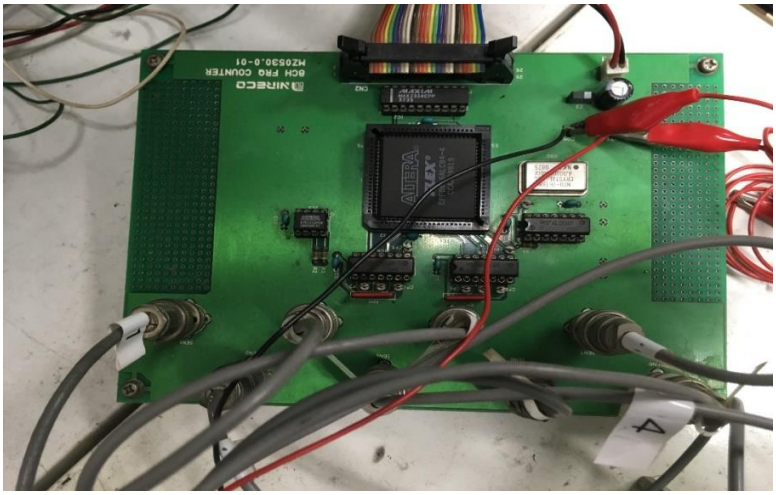


Fig. 3-5 Frequency counter used in this study

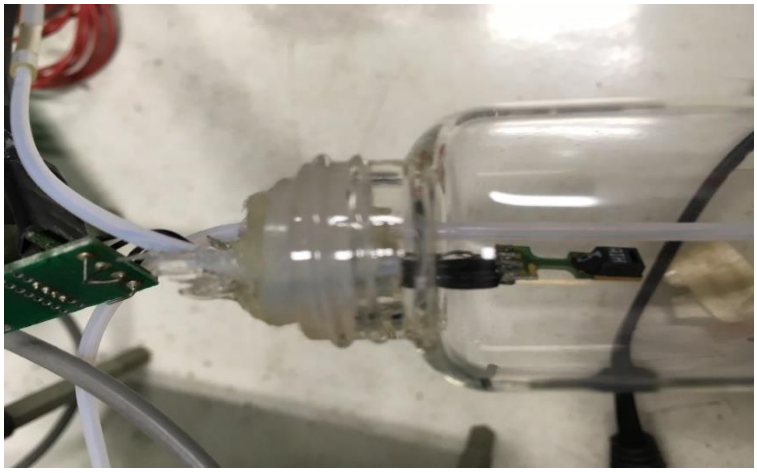

Fig. 3-6 Humidity sensor contained in the bottle

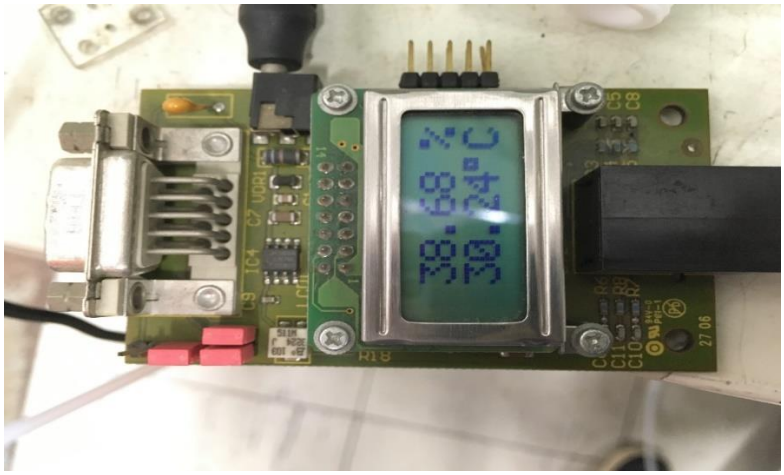

Fig. 3-7 Circuit board connected to the humidity sensor

The humidity sensor (SHT7x, Sensirion Inc) is set at the exit of the sensor chamber to observe the humidity of the sample gas. It is contained in a bottle as shown in Fig. 3-6. It is connected to a circuit board that enables us to read the humidity and temperature as shown in Fig. 3-7. We are not able to get the data of the humidity sensor by computer. By letting the air go through the bubbler contained with water, we have measured the humidity and see the maximum humidity. Here only one MFC was used. We set the flow rate at 200SCCM for dry air. Every 20 minutes we recorded the data as shown in Fig. 3-8. We can see that it took about 2 hours to reach about 80% RH. And the maximum humidity we can get at 200SCCM was about 85%.

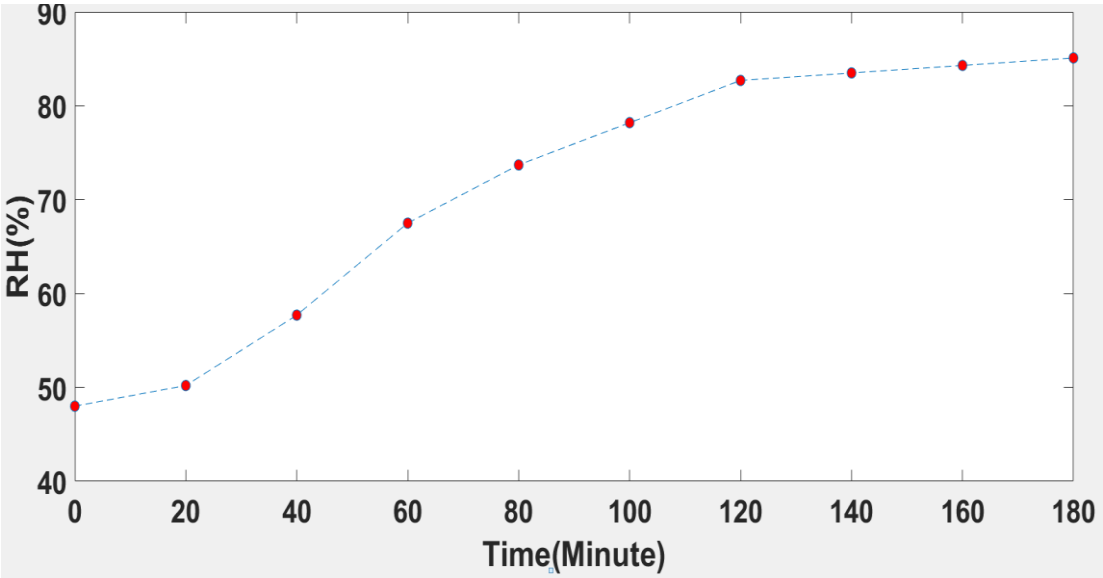


Fig. 3-8 Humidity change at the flow rate of 200SCCM

3.2 Improvement of measurement system

To control the relative concentration of the sample gas more conveniently, the odorant blending system was improved.

A solenoid valve controlling board for the odorant blending system was newly built in this study. It is made up of USB-Parallel conversion module FT-245RL, several IC chips and zener diodes. Fig. 3-9 shows the circuit of solenoid valve controlling board. We send the 8-bit serial signal from computer to the board. FT-245RL converts the serial signal to the parallel signal. Since pairs of solenoid valves work complementarily, the inverter chip was added. By setting the duration time of the solenoid valves, we can control the relative concentration of the sample gas.

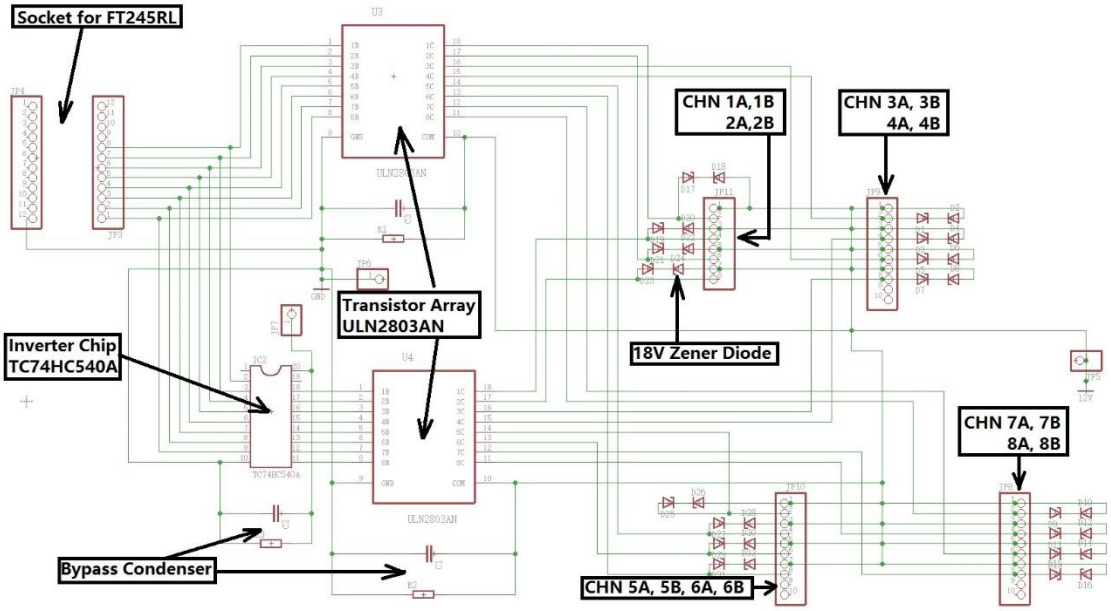


Fig. 3-9 Schematic of solenoid valve controlling board

With the solenoid valve controlling board, we are able to send signal from Matlab and realize controlling the relative concentration of the sample automatically. In Matlab, we configure the serial communication including the port, baud rate. And we can use the function in Matlab such as pause function, timer function to control the signal sent to FT245RL. The signal can be set at the resolution of 1ms. But it will occur delay in communication, so the exact resolution of time setting is about 20-30ms.

## 3.3 Summary

In this chapter, we introduced the schematic of the measurement system and what's new in the measurement system.

# Chapter 4 Testing and results

## 4.1 Coating of sensing film

### 4.1.1 Siponate DS-10

To compare with OBP, we used Siponate DS-10 which is the stationary phase material of gas chromatography. We used the ultrasonic atomizer to atomize the chloroform solution which dissolves DS-10. The atomizer was used for fabrication of QCM sensors by means of depositing thin organic films on the surface of the QCM device. Atomizer consists basically of (i) deposition chamber, (ii) atomizing stage (flask, ultrasonic device and the water tank), (iii) tubing. The procedure shows as follows:

1. Briefly, before the coating we should make sure the system is clean. We clean the flask by rinsing it twice-thrice with chloroform after which we run the system with chloroform only (ca. 1mL) at a flow rate of ca. 50mL/min until it disappears from the flask.
2. The sensors to be coated should be measured (frequency and resistance) prior to the fabrication. We use the network analyzer (MS4630B, Anritsu Inc.) to measure it.
3. Dissolve DS-10 in chloroform. The concentration of the solution is kept somewhere around 1g/L.
4. Once the solution is prepared, put around 1mL in the flask, fix the plug and the tubing. Put the sensor in the deposition chamber, make sure that we can see the curve on Network Analyzer display. Then close the chamber with the cover, connect the necessary tubes.
5. Start the process by switching the ultrasonic device on and applying the flow of gas. By observing the curve on Network Analyzer display, we should make adjustments if we are not satisfied with the speed of coating (flow rate, position of the QCM inside the chamber, position of the nozzles against the QCM surface).
6. Take the sensor out from the chamber and measure it again with network analyzer 1-2 hours after coating.

There are some points to bear in mind:

1. We must make sure the ultrasonic device is covered with water before we switch it on.

2. We must make sure the ultrasonic device is off when we are finished coating.
3. We must keep the system clean, rinse it frequently with chloroform. At least rinse it before coating.

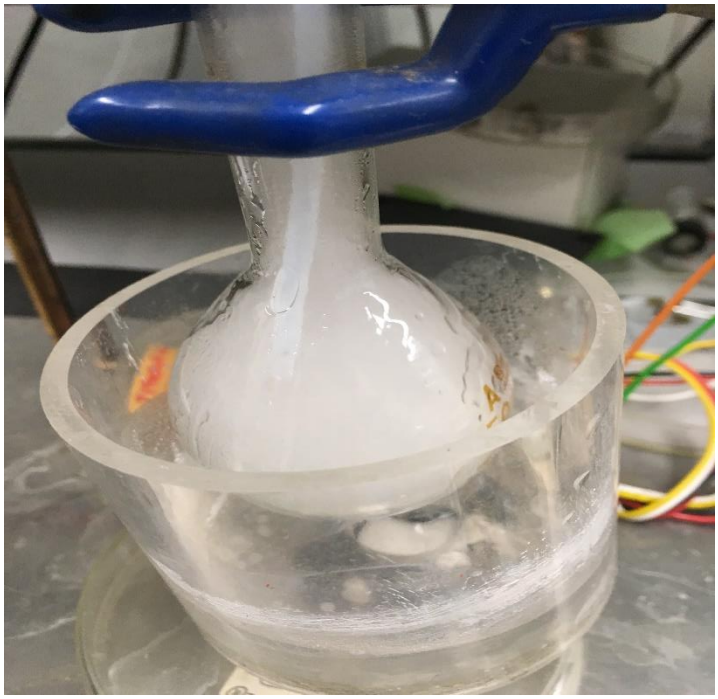

Fig. 4-1 Atomization in a flask

We have coated 1 QCM sensor with Siponate DS-10. Fig. 4-1 shows that the chloroform solution is being atomized. The result of DS-10 coating is shown together with OBP.

### 4.1.2 OBP

Three QCM sensors were individually coated with A.gam OBP4, A.gam OBP1 and PiOBP1. When OBPs were coated, we used the method of covalent immobilization – self assembled monolayer (SAM) to complete the coating. The procedure is shown in Chapter 2.2.

Table.1 shows the resonance frequency of the QCM sensors (AT-Cut, 30MHz) before and after coating. In each method, the resonance frequencies of the dried sensors were measured before and after the deposition process using a network analyser (Anritsu, MS4630B). We can see that the coating amounts of DS-10, A.gam OBP4 and Pig OBP1 were

similar.

Table 1. Resonance frequency of the QCM sensors before and after coating

| | Before Coating(MHz) | After Coating(MHz) | Frequency Shift(Hz) |
|---|---|---|---|
| DS-10 | 29.998600 | 29.992365 | 6235 |
| A.gam OBP1 | 30.064000 | 30.032296 | 31704 |
| A.gam OBP4 | 30.019600 | 30.014098 | 5502 |
| Pig OBP1 | 29.972600 | 29.968938 | 3662 |

4.1.3 OR

The cells expressing two kinds of ORs were used in this experiment. They were Or56a and Or13a cultivated continuously by Mr. Sukekawa in our lab.

We dropped ringer's solution including cells on both surfaces of the QCM sensors and measured the resonance frequency before and after coating. The resonance frequency was measured using the network analyser. It is shown in Table.2.

Table.2 Resonance frequency of the QCM sensors before and after coating

| | Before Coating(MHz) | After Coating(MHz) | Frequency Shift(Hz) |
|---|---|---|---|
| Or56a | 9.000717 | 8.996136 | 4581 |
| Or13a | 9.000269 | 8.996961 | 3308 |

4.2 Testing condition

4.2.1 OBP

In the experiment with QCM sensor coated with DS-10 and OBP, before the measurement, the air was flowed at 100ml/min through the chamber until the stable baselines within 2Hz variation were obtained. In this study, it takes 180 seconds before each measurement.

Then, the sample gas with the relative concentration of 10% was supplied to the measurement system for 60s. Then, we supplied the air to

recover the sensors. We measured each sample three times. Fig. 4-2 shows the sequence of experiment.

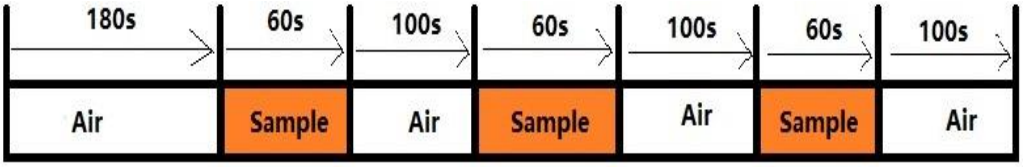


Fig. 4-2 Sequence of experiment (OBP)

First we tested 4 sensors mentioned with the samples at the dry-air condition. After the initial measurement, the responses of the sensors were found to be too large. We tried to suppress the response by diluting the sample with ODO (Octyl Decil Oil) which is a kind of odourless non-volatile liquid because the sensor responses might be saturated. The samples with the dilution ratios of 50%, 25%, 16% were measured.

Moreover, we increased the relative humidity to about 70% and tested the sensors with the same procedure.

### 4.2.2 OR

The samples we used in this experiment are geosmin, 1-octen-3-ol, benzaldehyde and amyl acetate. It is known from the biological experiment that Or56a shows sensitivity to geosmin and Or13a shows sensitivity to 1-octen-3-ol. Benzaldehyde and amyl acetate were used for comparison.

Before the measurement, the air was flowed at 200ml/min through the chamber until the baseline becomes stable. We changed the flow rate from 100ml/min to 200ml/min since the sensors did not recover completely in the experiment of OBP sensors. In this study, it takes about 600 seconds before each measurement.

Then, the sample gas with the relative concentration of 100% was flowed to the measurement system for 30s. Then, we flowed the air to recover the sensors for 70s. We measured each sample three times. The experiment sequence is shown in Fig. 4-3.

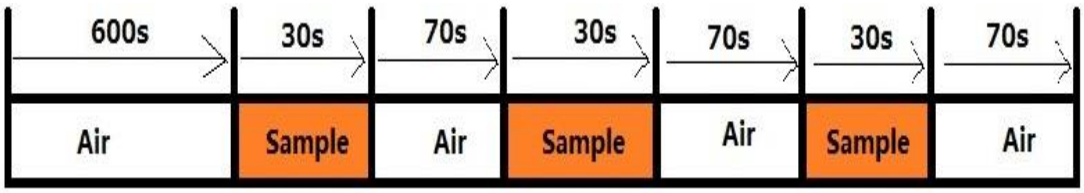


Fig. 4-3 Sequence of experiment (OR)

In the initial experiment, geosmin and 1-octen-3-ol without dilution were used. We found the responses of the sensors were too large and it also had the possibility of the sensor response saturation. To suppress the response, we diluted the sample with ODO. Geosmin was diluted with the dilution ratio of 10%, 1% and 0.1%. 1-octen-3-ol was diluted with the dilution ratio of 10% and 1%. We measured the sensor response at each dilution ratio.

To see the relationship of sensor response and relative concentration, we decreased the relative concentration from 100% to 20% at a step of 20%.

Relative concentration in the software was 100% except Fig. 4-12~4-14.

To confirm whether the samples do not bind with the bare gold electrode, we tested the QCM sensors without coating at 70%RH.

Moreover, we set the relative humidity at about 50% and tested the sensors with the same procedure to see the relationship between sensor response and humidity.

We also investigated the life time of QCM sensors coated with cells expressing Or56a and Or13a. After we did the experiments mentioned before, the sensors were preserved in the ringer's solution. We did other experiments in the same procedure every 3-4 days.

## 4.3 Results of experiment of QCM sensors coated with OBP

The samples used here were benzaldehyde and amyl acetate since the OBPs were expected to respond to them. The maximum frequency change during the exposure was taken as a sensor response.

Fig. 4-4 and 4-5 show the response of Pig OBP1 sensor and A.gam OBP4 sensor before and after the dilution at different proportion, respectively.

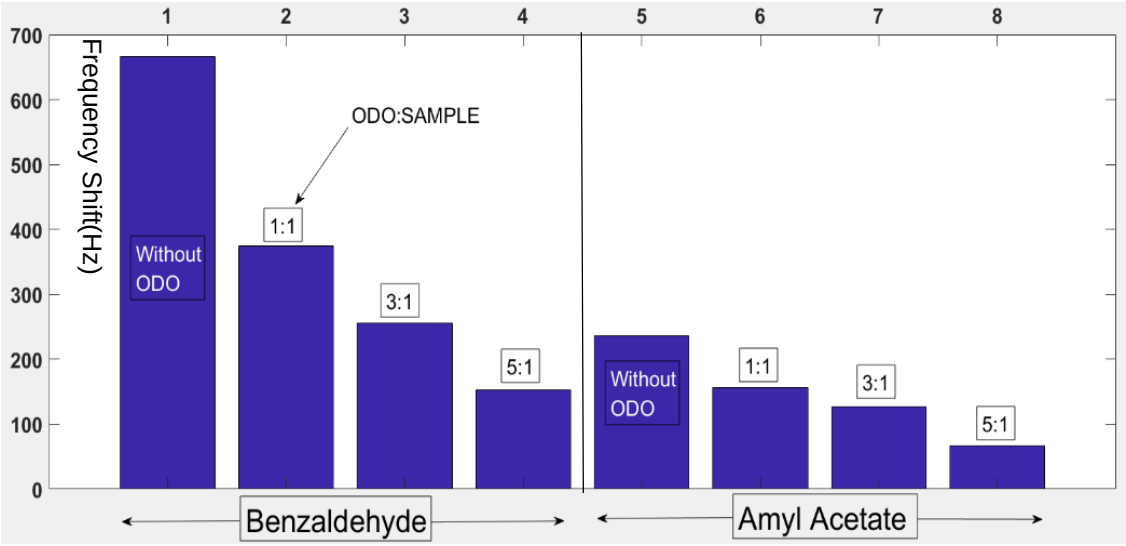


Fig. 4-4 Responses of Pig OBP1 sensor to two samples at different dilution proportions. (Humidity: 0% RH)

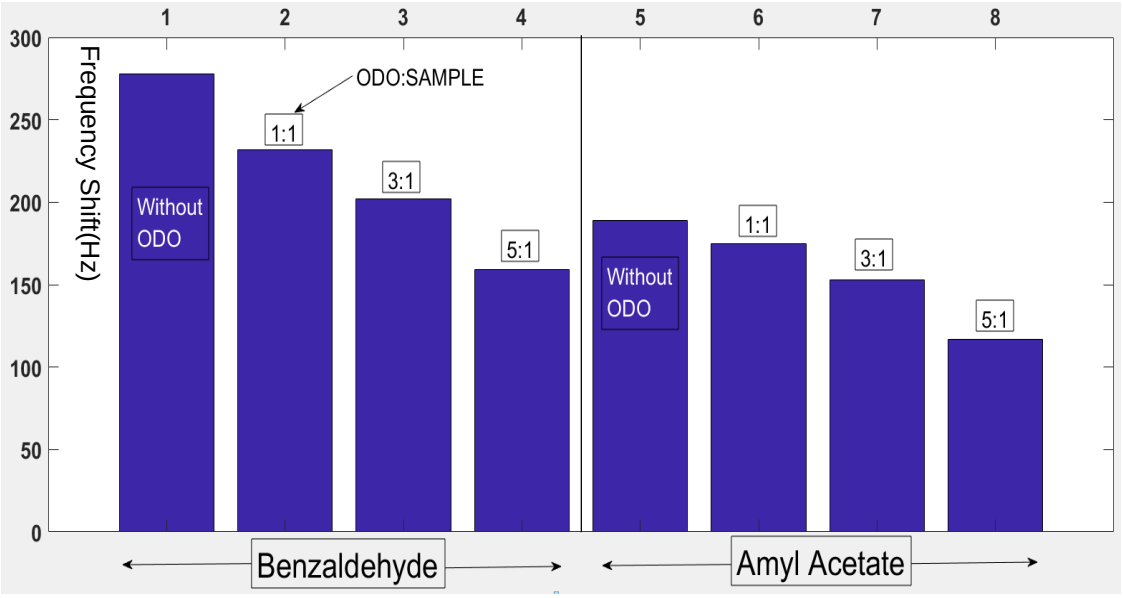


Fig. 4-5 Responses of A.gam OBP4 sensor to two samples at different dilution proportions. (Humidity: 0% RH)

From Fig. 4-4 and 4-5, we can see that the sensor responses decreased with the increase in dilution proportion. The dilution with ODO has effect on the frequency shift. Also the sensor with Pig OBP1 seems to be more sensitive to the dilution.

With the dilution proportion of 25%, we increased the relative humidity of

the experiment environment to about 70%. With the same procedure, we tested 4 sensors.

Fig. 4-6 and 4-7 demonstrate the responses of 4 sensors to 2 samples at 0% and 70% RH. Although the humidity environment was different, the response pattern was almost the same. Although the film thickness of DS-10, A.gam OBP4 and Pig OBP1 were similar, the responses of OBPs were larger than DS-10 with the same amount of coating. It was found that sensors coated with OBPs were more sensitive than sensors coated with DS-10. Although the amount of A.gam OBP1 coated was larger than A.gam OBP4, the response of A.gam OBP4 was larger. We consider that A.gam OBP4 was more sensitive to A.gam OBP1 even the humidity changed. Pig OBP1 seems to have the selectivity between the 2 samples.

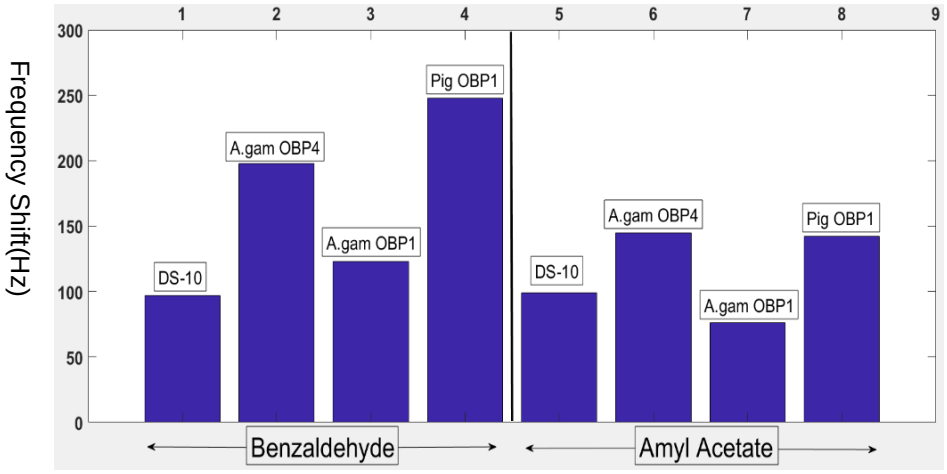


Fig. 4-6 Response of 4 sensors to samples at 0% humidity. (Dilution: 25%)

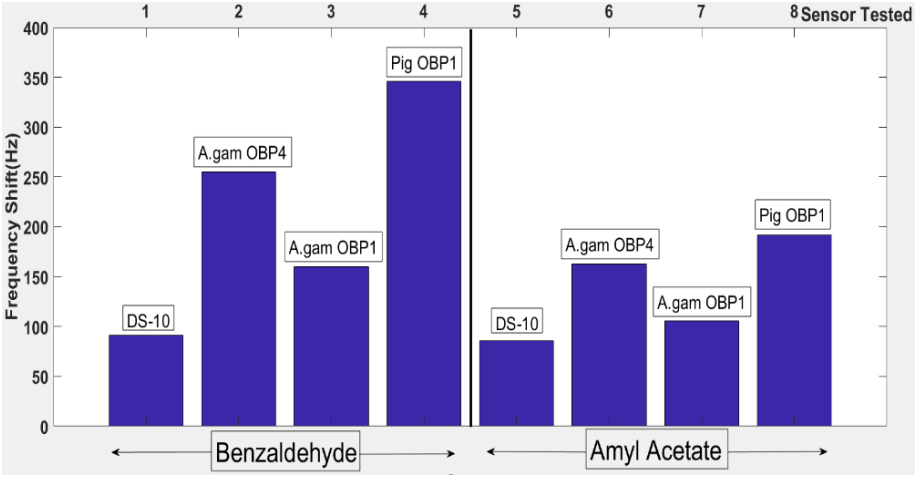


Fig. 4-7 Response of 4 sensors to samples at 70% humidity. (Dilution: 25%)

Fig 4-8~4-11 show the sensor response of 4 sensors to 2 samples (benzaldehyde, amyl acetate) individually at 0% RH and 70% RH.

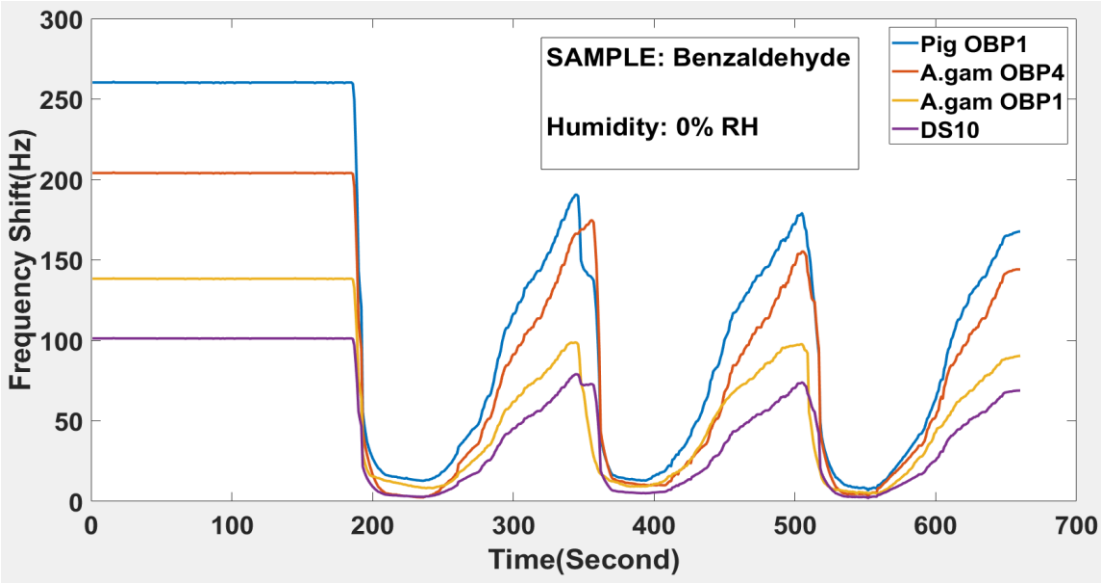


Fig. 4-8 Sensor response of 4 sensors to benzaldehyde at 0% RH

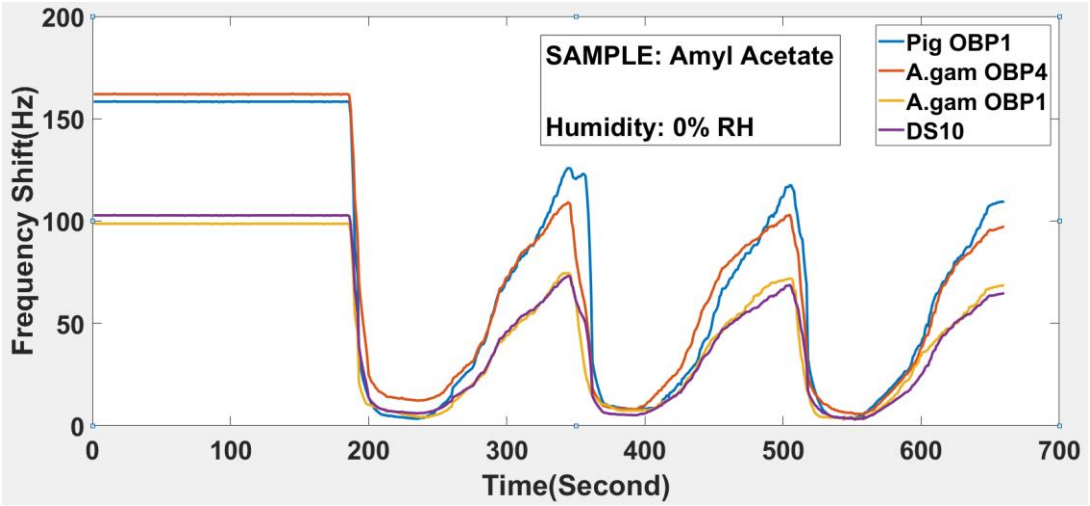


Fig. 4-9 Sensor response of 4 sensors to amyl acetate at 0% RH

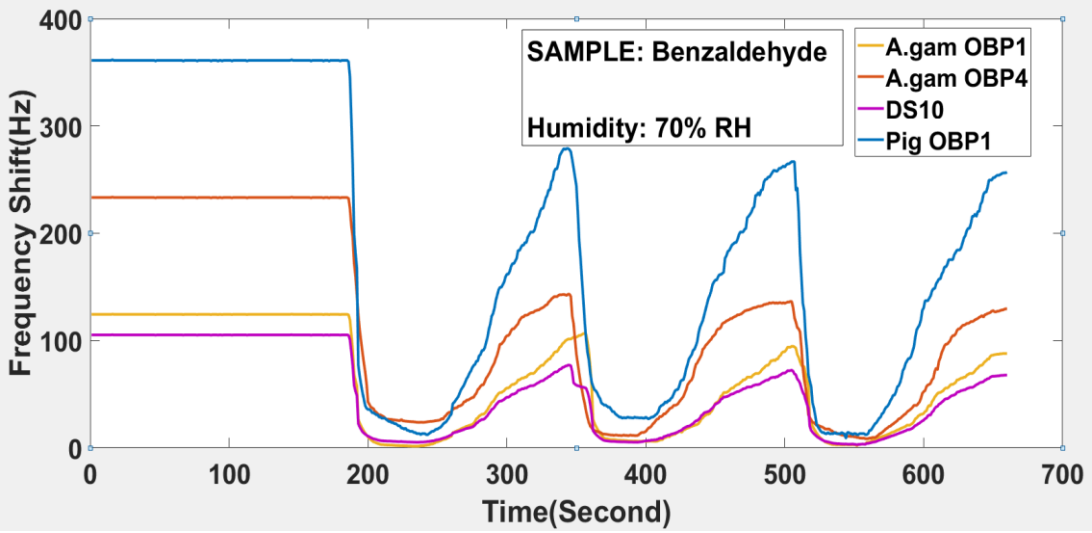


Fig. 4-10 Sensor response of 4 sensors to benzaldehyde at 70% RH

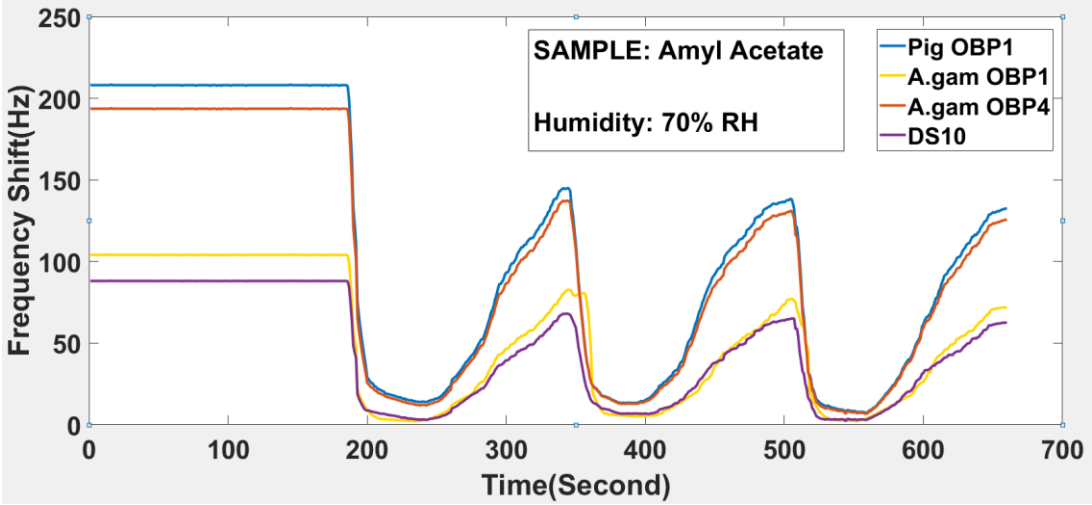


Fig. 4-11 Sensor response of 4 sensors to amyl acetate at 70% RH

Fig. 4-12 shows the comparison of the response of sensor coated with Pig OBP1 at 0% and 70% humidity respectively. In high humidity environment, the frequency shift increased whereas the response of Siponate DS-10 sensor decreased a little with the humidity level. It was found that high humidity may let the OBP be more sensitive.

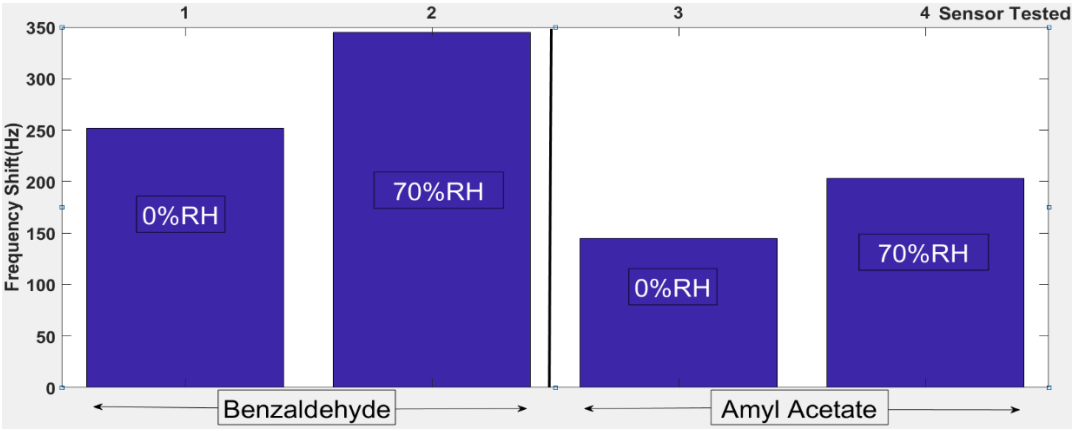


Fig. 4-12 Response of sensor coated with Pig OBP1 to sample at 0% and 70% humidity. (Dilution: 25%)

4.4 Results of experiments of QCM sensors coated with cell expressing OR

Fig. 4-13 and 4-14 show the dilution effect of ODO on the sensor response. In the case of Or56a, we tried dilution ratio of 10%, 1% and 0.1%. For the case of Or13a, we tried dilution ratio of 10% and 1%. It was seen that the more we diluted the sample with ODO, the smaller the sensor response was obtained. Dilution with ODO has the effect of suppressing the sensor response.

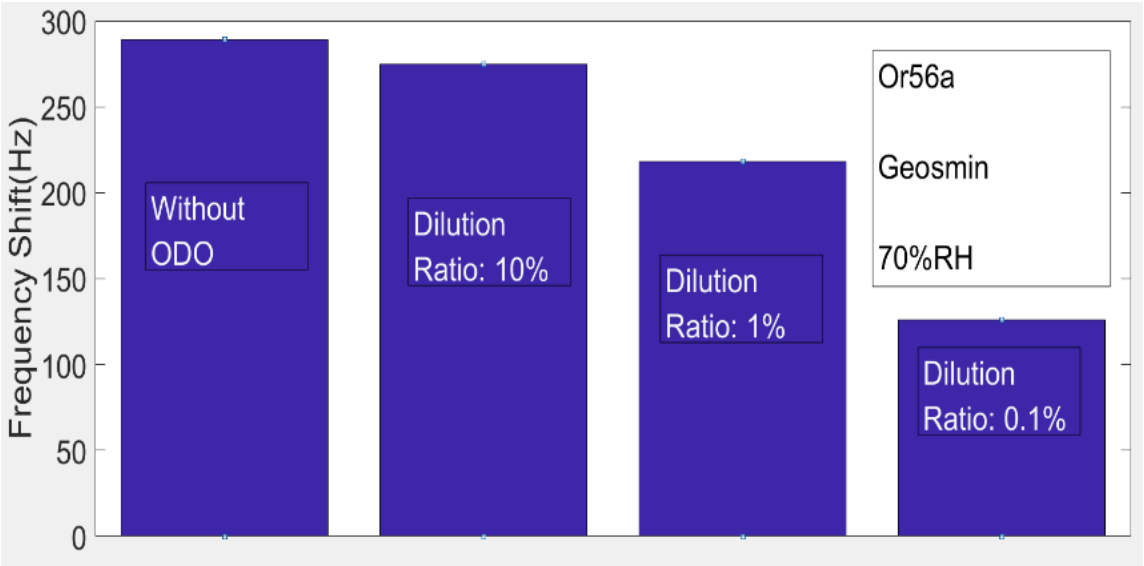


Fig. 4-13 Dilution effect of ODO on the response of QCM coated with cell expressing Or56a to geosmin

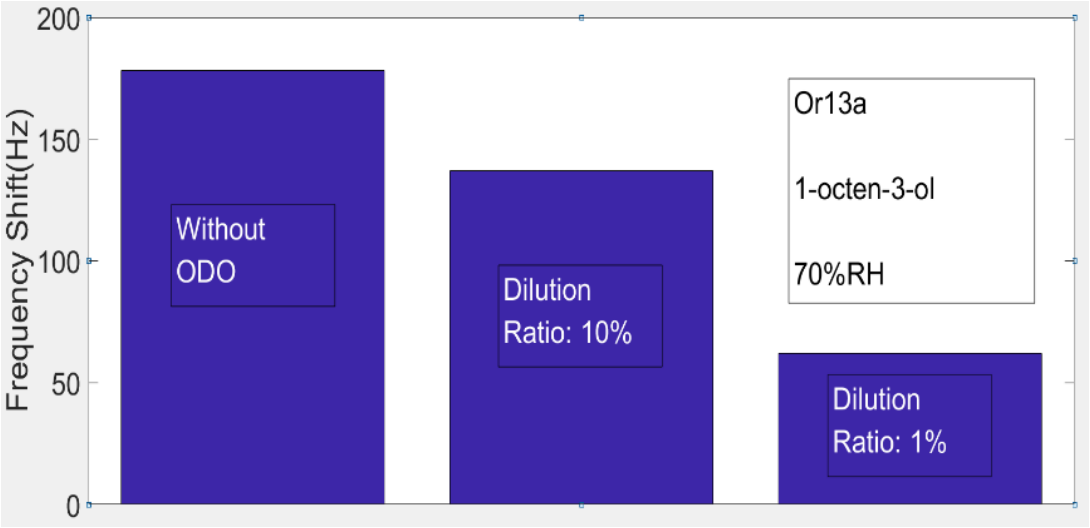


Fig. 4-14 Dilution effect of ODO on the response of QCM coated with cell expressing Or13a to geosmin

Fig. 4-15 shows the sensor response of QCM sensors coated with cells expressing Or56a and Or13a to 4 samples at 70%RH. The exact concentration of samples measured using PID detector PpbRAE 3000(RAE Systems.Co) are shown in the figure. Why the concentration of geosmin has a range is because that RAE Systems. Co does not have a precise correction factor for geosmin. We can see that for Or56a, its response to geosmin was larger than other samples despite its low gas concentration and the response of Or13a sensor to 1-octen-3-ol was larger than other samples. It was found that Or56a had the selectivity to geosmin and Or13a had the selectivity to 1-octen-3-ol even if they worked in the gas phase.

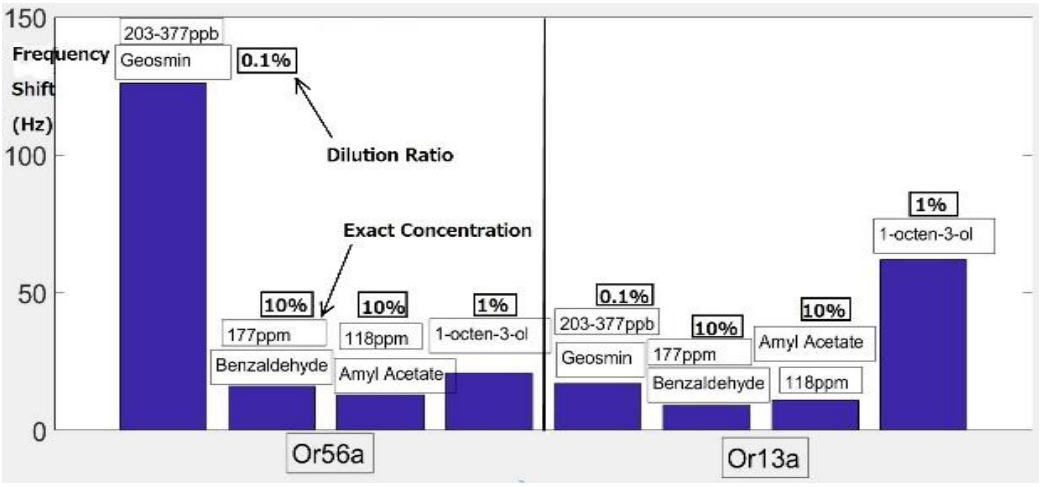


Fig. 4-15 Sensor response of QCM sensors coated with Or56a and Or13a to 4 samples at 70% RH

Fig. 4-16 shows the sensor response of QCM sensor coated with cell expressing Or56a to geosmin at 70% RH. Fig. 4-17 shows the sensor response of QCM sensor coated with cell expressing Or13a to 1-octen-3-ol at 70% RH.

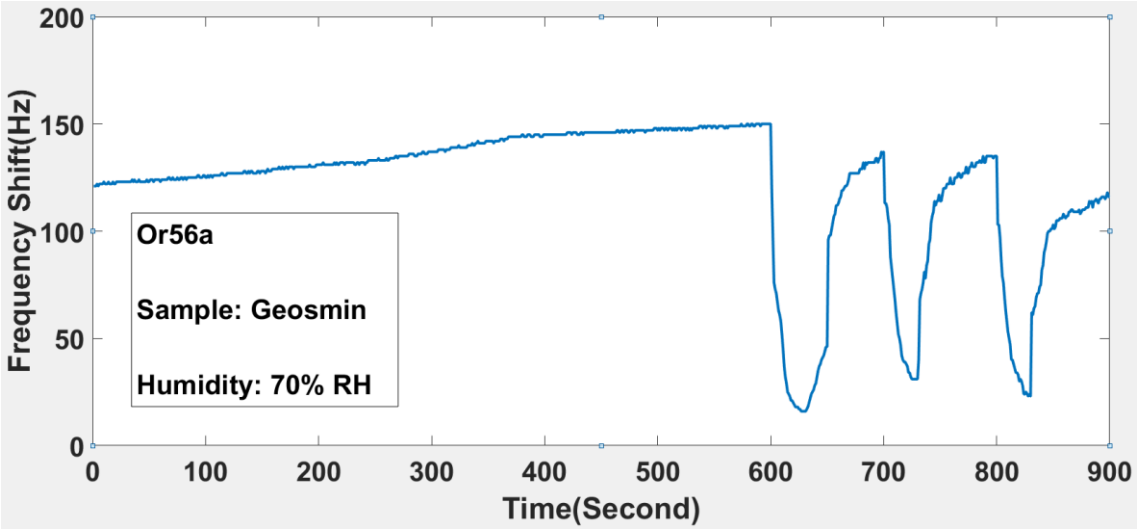


Fig. 4-16 Sensor response of QCM sensor coated with cell expressing Or56a to geosmin at 70% RH

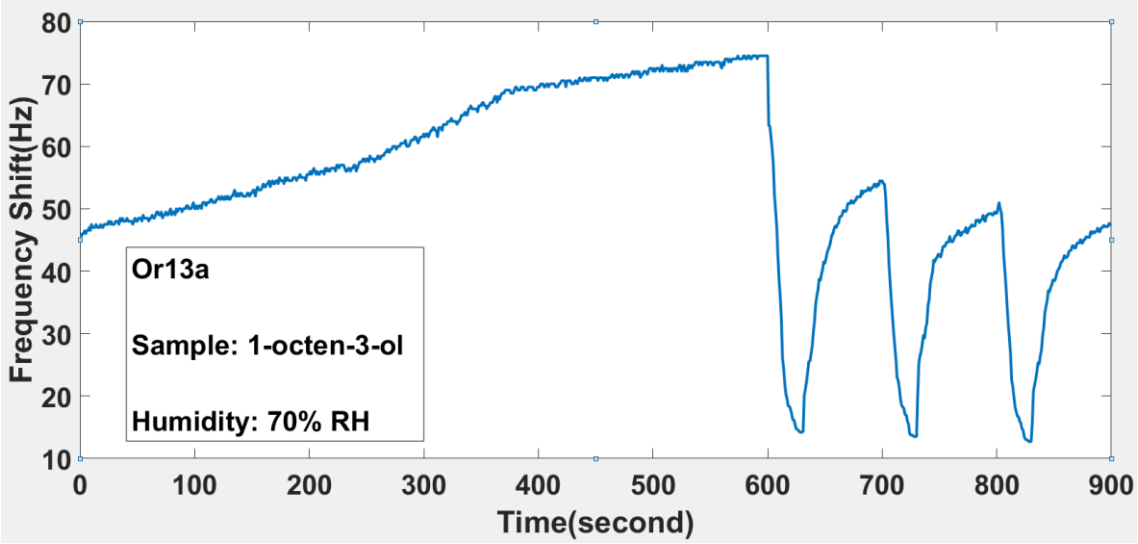


Fig. 4-17 Sensor response of QCM sensor coated with cell expressing Or13a to 1-octen-3-ol at 70% RH

Fig. 4-18 and 4-19 show the relationship between sensor response of Or56a sensor to geosmin and relative concentration of sample gas at the dilution ratio of 10% and 0.1%. In Fig. 4-18, though we decrease the relative

concentration of geosmin from 100% to 20% at the step of 20%, the sensor response only decreased from about 270Hz to about 220Hz. It had a large possibility of sensor response saturation at the dilution ratio of 10%. When the dilution ratio was 0.1% in Fig. 4-19, though the relationship between concentration and response was not completely linear, it was similarly linear. We considered the saturation almost disappeared at the dilution ratio of 0.1%.

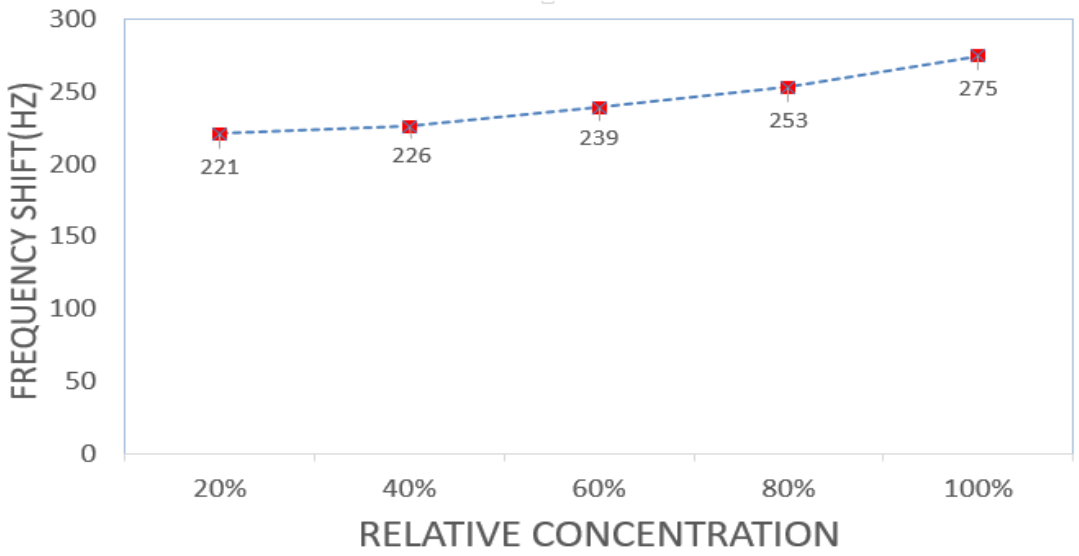


Fig. 4-18 Relationship between sensor response of Or56a to geosmin and relative concentration of sample gas at the dilution ratio of 10% (Humidity: 70% RH)

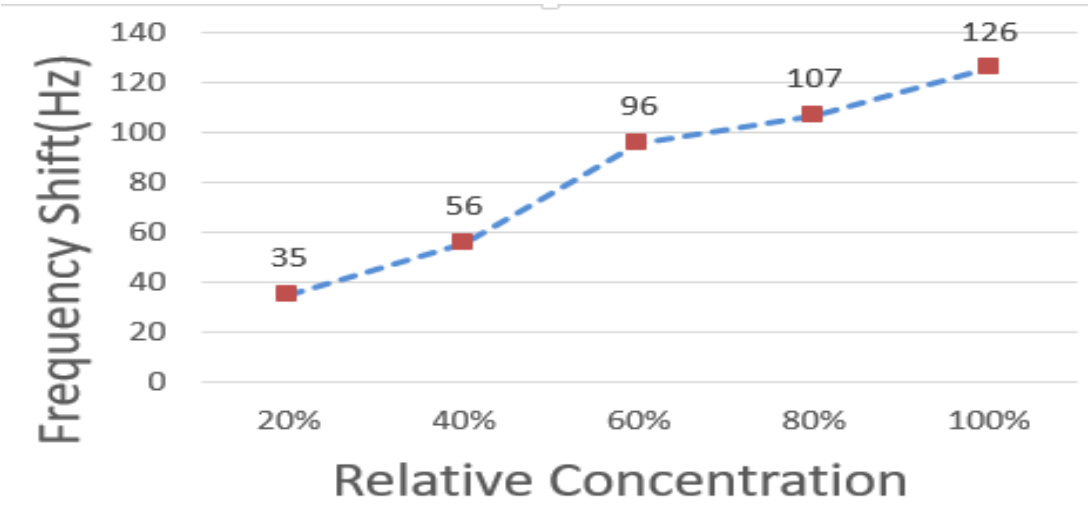


Fig. 4-19 Relationship between sensor response of Or56a to geosmin and relative concentration of sample gas at the dilution ratio of 0.1% (Humidity: 70% RH)

Fig. 4-20 shows the relationship between response of Or13a sensor to 1-octen-3-ol and relative concentration of sample gas at the dilution ratio of 1%. It has the similar result with Fig. 4-19.

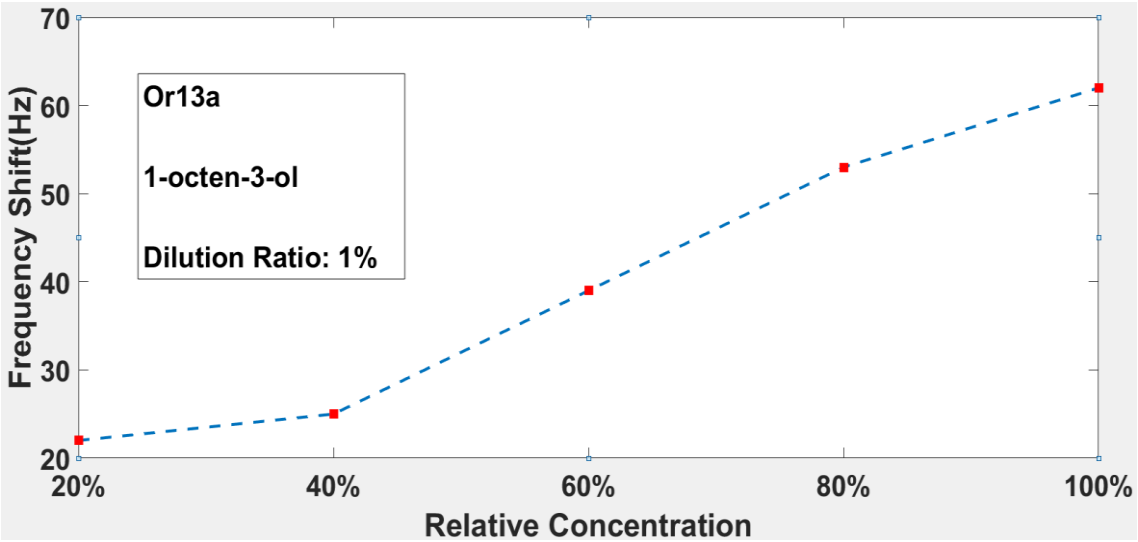


Fig. 4-20 Relationship between sensor response of Or13a sensor to 1-octen-3-ol and relative concentration of sample gas at the dilution ratio of 1% (Humidity: 70% RH)

Fig. 4-21 and 4-22 show the humidity experiment of Or56a and Or13a sensors. We can see both sensor responses decreased when relative humidity decreased from 70% to 50%. It was found that the sensors are more sensitive in higher humidity environment.

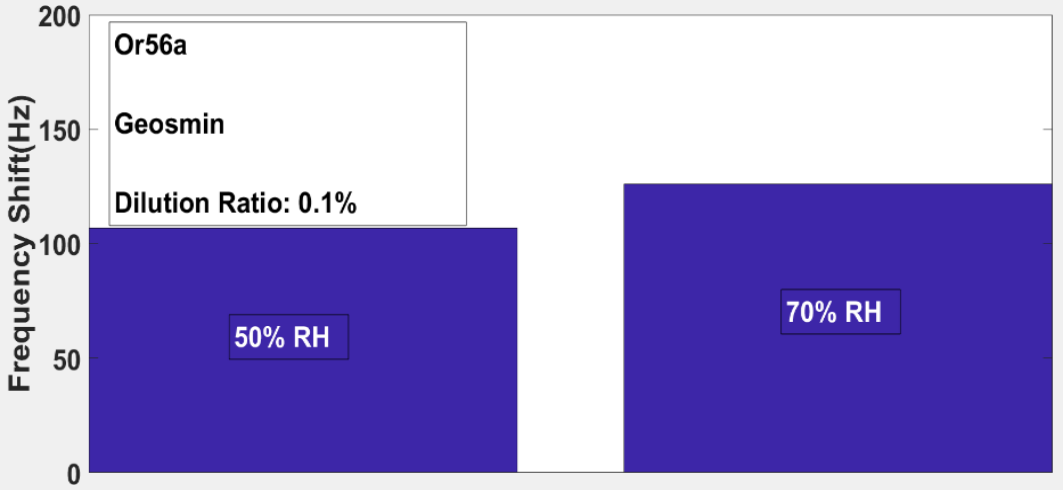


Fig. 4-21 Sensor response of Or56a sensor to geosmin under 70%RH and 50%RH

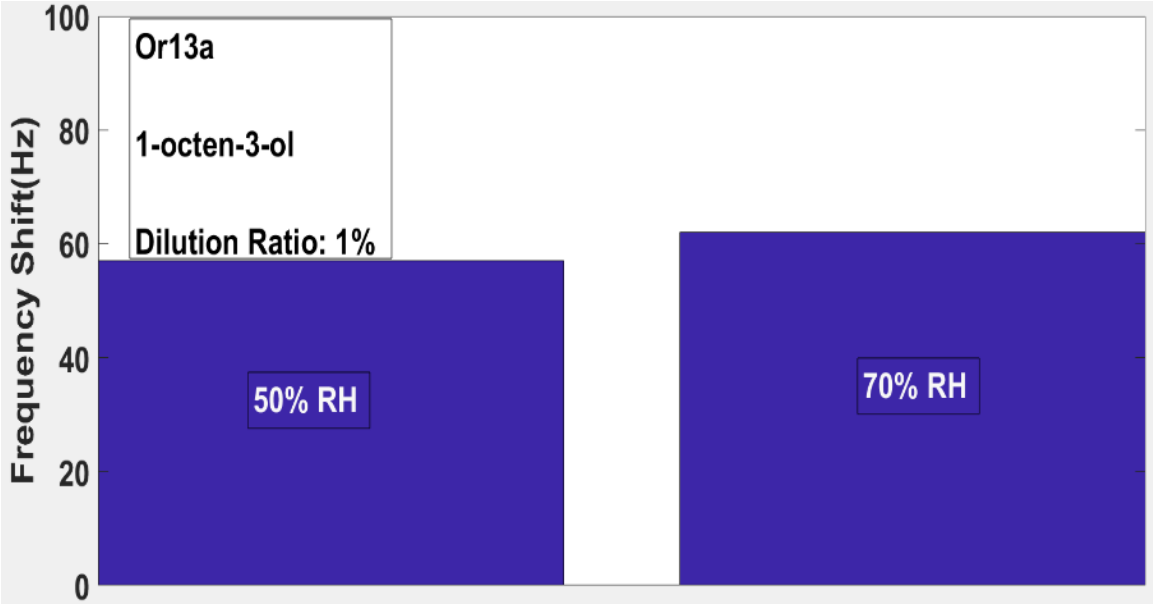


Fig. 4-22 Sensor response of Or13a sensor to 1-octen-3-ol under 70%RH and 50%RH

Fig. 4-23 shows the response of QCM sensors without coating to 4 samples. The responses of bare gold film on QCMs were all small. We confirmed that the odorant molecule of samples do not have bind with the gold electrode and the sensor response in Fig. 4-15 comes from olfactory receptor cells coated on QCM sensors.

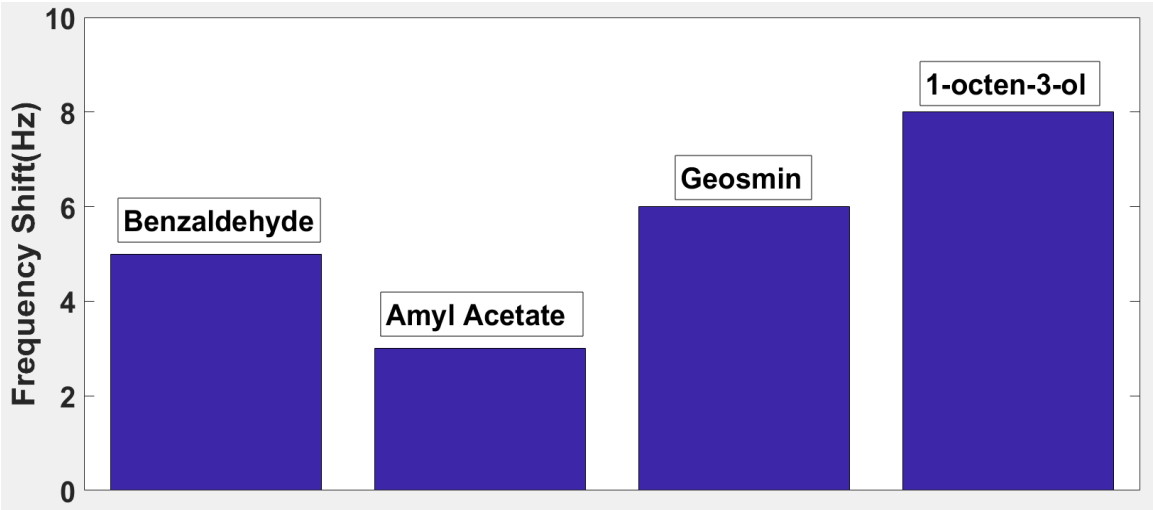


Fig. 4-23Response of QCM sensors without coating to 4 samples

Fig. 4-24 and 4-25 show the result of life time experiment. The same experiment was performed every 3-4 days. Initial experiment was 5 days after the cultivation. Fig. 4-24 shows the result of Or56a and Fig. 4-25 shows the result of Or13a. For Or56a, though the response decreased little by little, it still had sensitivity and selectivity after about 15 days since the cultivation. For Or13a, in the last experiment, the response decreased about half. Fig. 4-26 shows the frequency shift of baseline of 2 sensors after the initial experiment day. The frequency shift occurred since the cells peeled off from the surface of the QCM sensors. We considered the duration time of Or56a is longer than that of Or13a. Moreover, the sensor worked longer than the life time of the cell (7-10days).

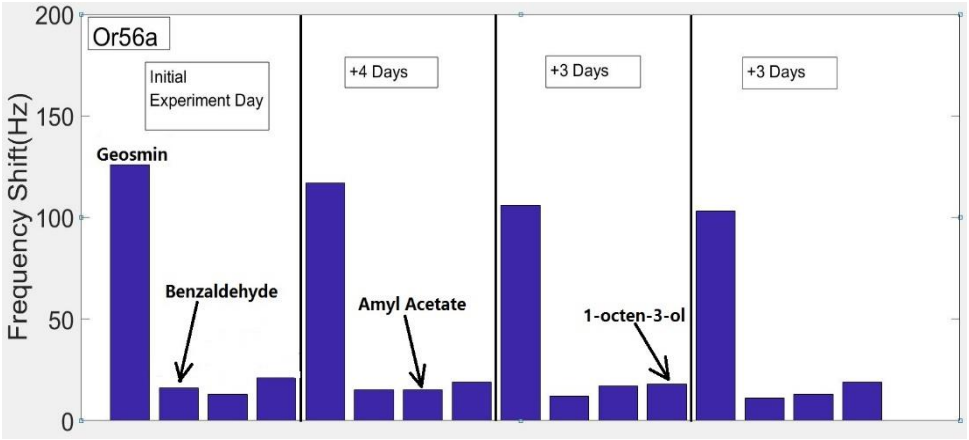


Fig. 4-24 Life time experiment of Or56a at 70% RH (preserved in beaker contained with water)

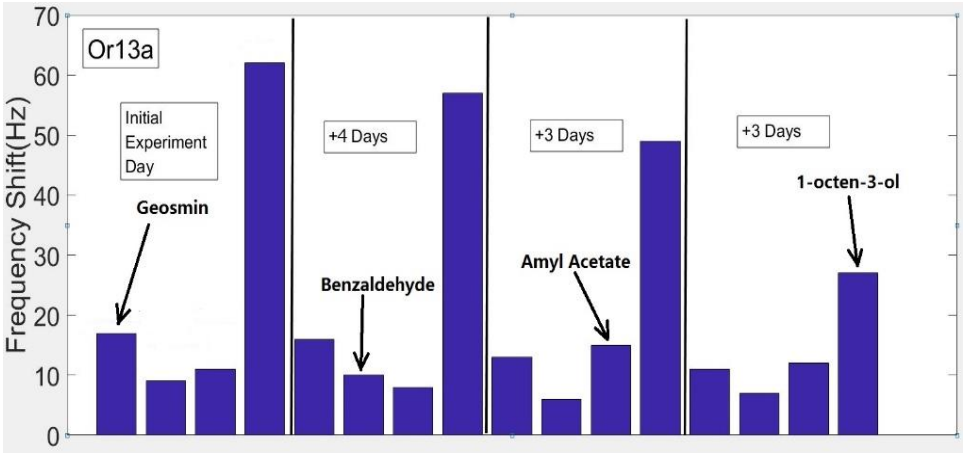


Fig. 4-25 Life time experiment of Or13a at 70% RH (preserved in beaker contained with water)

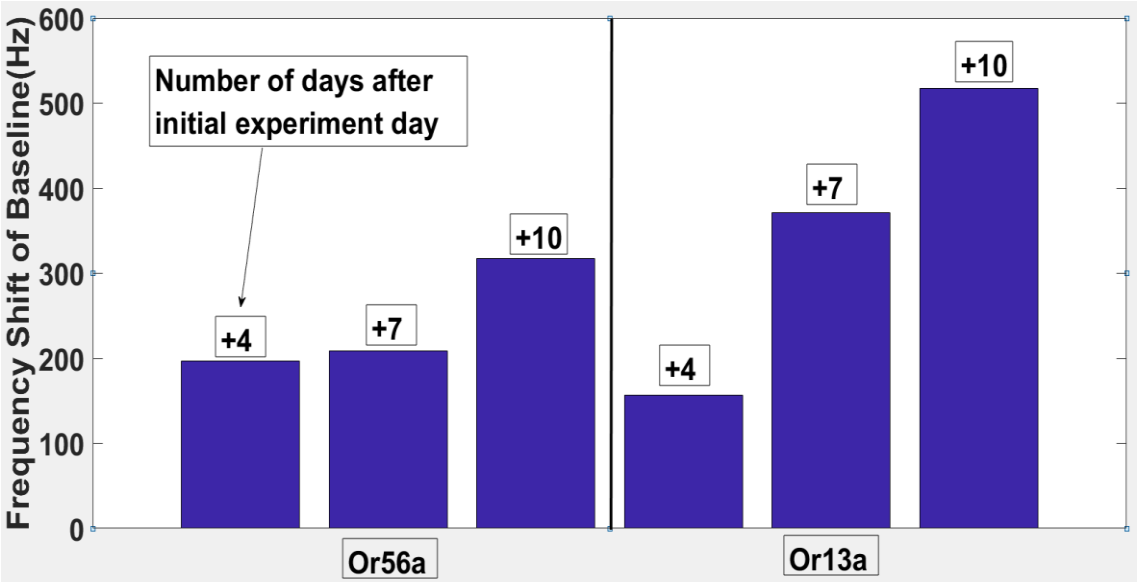


Fig. 4-26 Frequency shift of baseline of 2 sensors after the initial experiment day

4.5 Additional experiments of QCM sensors coated with cell expressing OR

We have done some additional experiment as the continuation of the life time experiment of OR. In the life time experiment of OR in the previous section, every time after the experiment, we took the sensors from the sensor chamber and preserved the sensors in the beaker contained with ringer's solution to supply the high humidity environment. From Fig. 4-26, we can see that this preservation method may cause the peeling of the OR. So we tried another preservation method. After every measurement, we did not take the sensors out of the sensor chamber. We maintained the sensor chamber at the high humidity environment (about 80% RH) continuously.

The frequency shift of 2 sensors before and after coating is shown in Table.3.

Table.3 Resonance frequency of the QCM sensors before and after coating

| | Before Coating(MHz) | After Coating(MHz) | Frequency Shift(Hz) |
|---|---|---|---|
| Or56a | 9.000167 | 8.998176 | 1991 |
| Or13a | 9.000031 | 8.997872 | 2159 |

Fig. 4-27 shows the result of life time experiment of Or56a and Fig. 4-28 shows the result of life time experiment of Or13a. We can see that compared with the previous preservation method, using the new preservation method has the possibility of extending the life time of OR.

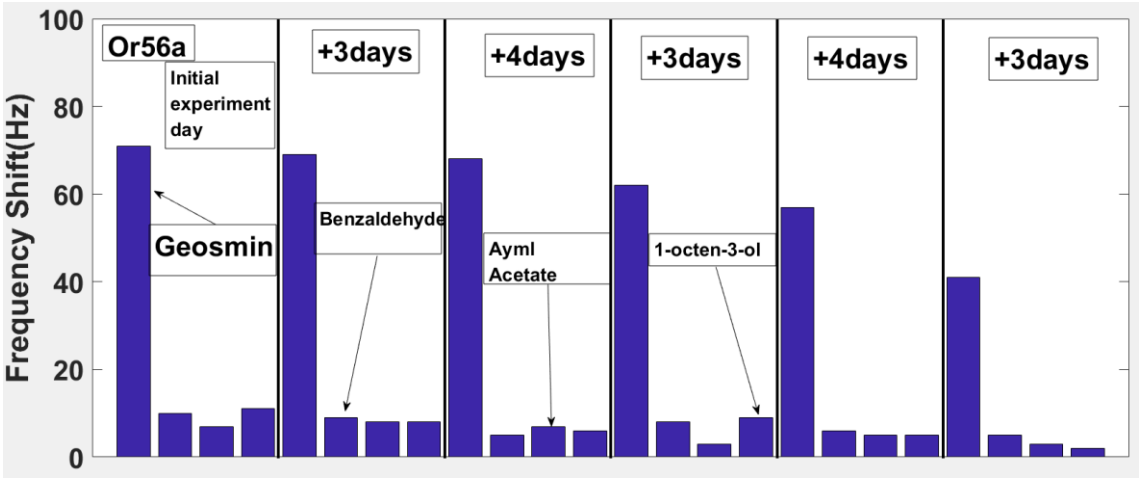


Fig. 4-27 Life time experiment of Or56a (sensors preserved in the sensor chamber)

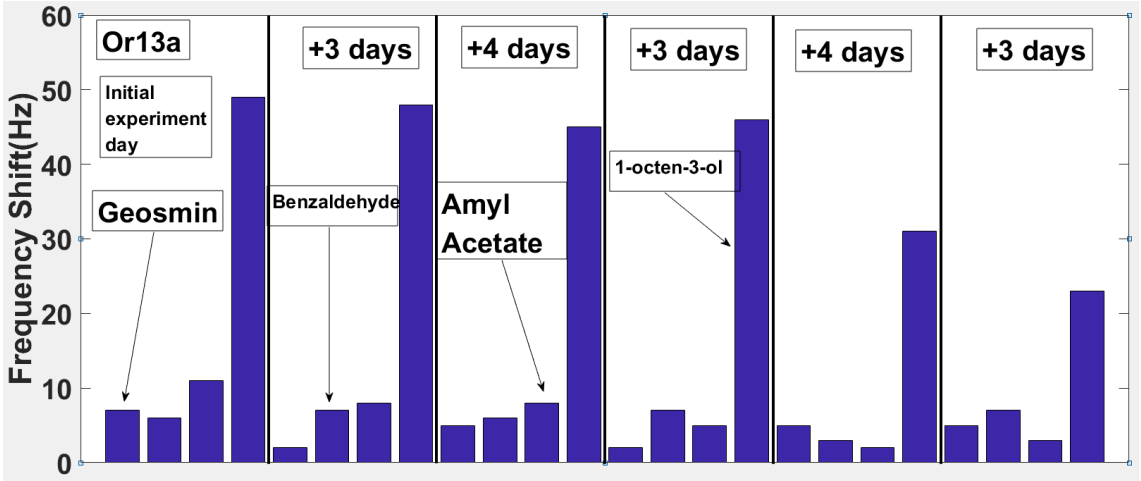


Fig. 4-28 Life time experiment of Or13a (sensors preserved in the sensor chamber)

Fig 4-29 shows the frequency shift of baseline of 2 sensors after the initial experiment day. We can see that for Or56a, the baseline frequency just changed a little. But for Or13a, the baseline frequency change largely in the final 2 experiment days. We consider the reason why Or56a sensor's response decreased was that Or56a's structure might be deformed. But the reason why Or13a sensor's response decreased was that the cells may have peeled from the QCM surfaces.

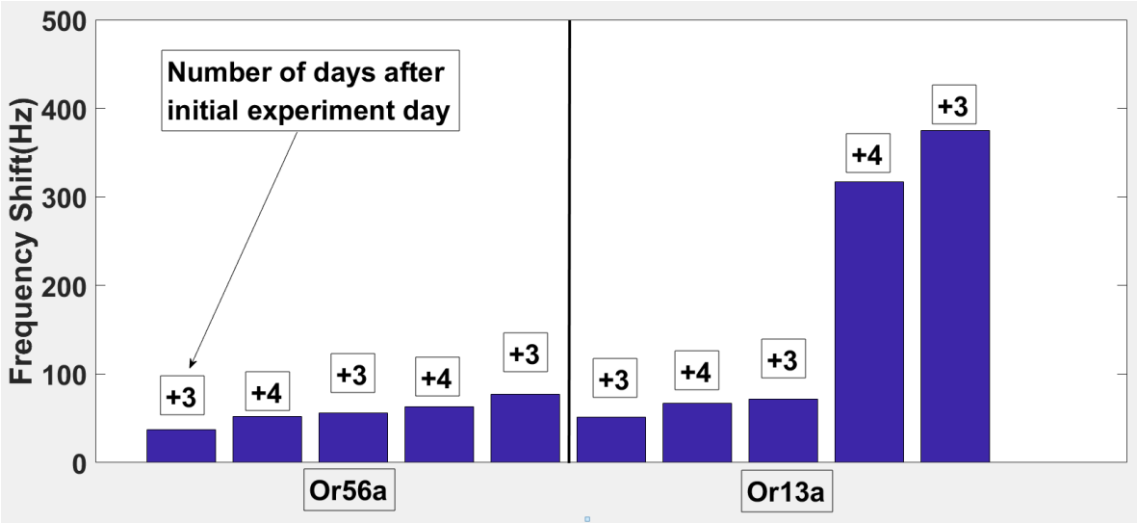


Fig. 4-29 Frequency shift of baseline of 2 sensors after the initial experiment day

To see whether QCM sensors coated with cells expressing OR can work in dry environment, we did another experiment. After coating 2 QCM sensors individually with Or56a and Or13a, we put them into the sensor chamber where the humidity has been maintained with about 80%. After getting the sensor response with the sequence of odor sample 30 seconds and air 70 seconds, we let the dry air go through the sensor chamber and measured the sensor response every 20 minutes 5 times. Table.4 shows the coating information of 2 sensors.

Table.3 Resonance frequency of the QCM sensors before and after coating

| | Before Coating(MHz) | After Coating(MHz) | Frequency Shift(Hz) |
|---|---|---|---|
| Or56a | 9.000671 | 8.997279 | 3392 |
| Or13a | 9.000362 | 8.998291 | 2071 |

Fig.4-30 and 4-31 show the response of QCM sensor coated with the cells expressing Or56a and Or13a. We can see that even the humidity has decreased to about 20%RH, the sensors still can have the sensor response. But we consider that it is hard for the sensors to maintain the response pattern for longer than 2 hours when the dry environment is supplied. And we consider that after the coating of cells expressing OR, touching in the atmosphere for a short time will not have large influence on the sensor property.

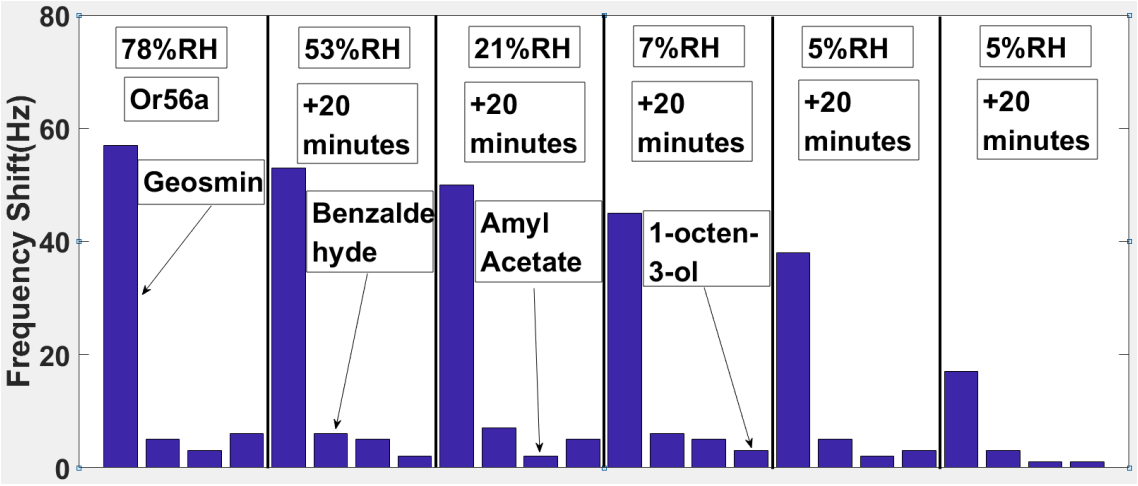


Fig. 4-30 Response of QCM sensor coated with the cell expressingOr56a

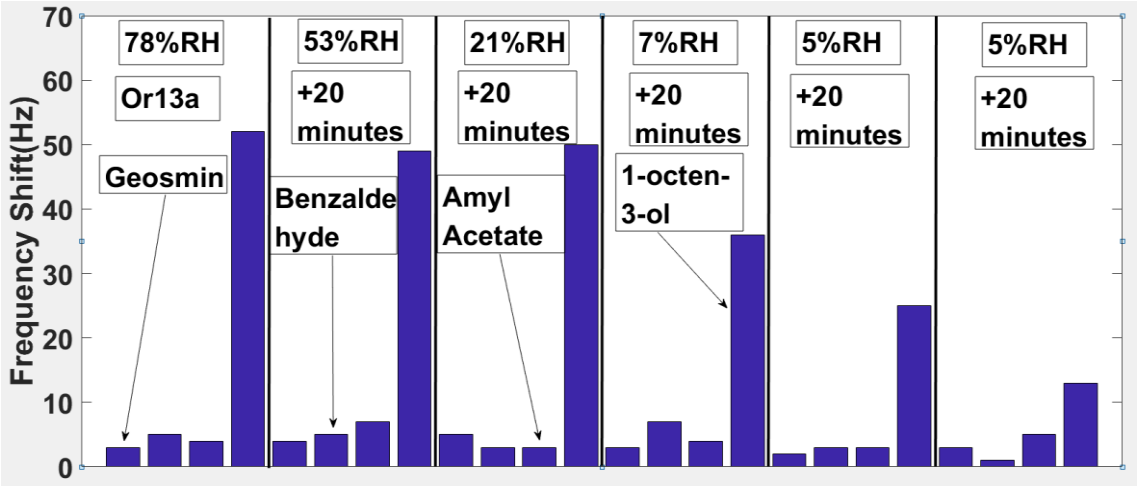


Fig. 4-31 Response of QCM sensor coated with the cell expressingOr13a

## 4.6 Summary

This chapter shows conditions and results of the experiment of QCM sensors coated with OBP and cells expressing OR. Furthermore, the additional experiment about the QCM coated with cells expressing OR is discussed in this chapter.

# Chapter 5 Conclusion

## 5.1 Achievements in this study

We had 3 achievements in this study.

First, we improved the measurement system. We embedded the solenoid valve controlling board into the measurement system. The system can automatically control the sequence to change the relative concentration of sample gas and measure the response in gas phase.

Second, an OBP was found to have potential for odor sensing. QCM sensors coated with OBPs were more sensitive than the QCM sensors coated with typical chemical sensing film such as Siponate DS-10 with the same amount of coating. A.gam OBP4 has better sensitivity than A.gam OBP1. Pig OBP1 is considered to have the selectivity between benzaldehyde and amyl acetate from the comparison of the frequency shift. High humidity may enhance the sensitivity of OBP to the odorant sample.

Third, in the experiment of QCM sensors coated with the cell expressing OR, it was found that Or56a had the selectivity with geosmin and Or13a had the selectivity with 1-octen-3-ol even in the gas phase. High humidity may also enhance the sensitivity of OR to the odorant sample. The life time of QCM sensors coated with Or56a and Or13a could be longer than 10 days. QCM sensors coated with Or56a is considered to have longer duration time than that coated with Or13a. Preserving the QCM sensors coated with cells expressing OR in the sensor chamber maintained with high humidity environment can extend the life time of such sensors compared with preserving the sensors in the beaker contained with water. And such sensors can have the sensor response at dry environment but not for a long time.

## 5.2 Future work

We had only confirmed the possibility of QCM sensors coated with OBP or OR as odorant sensor in the gas phase. Whether this kind of sensor has practical application, much work need to be done. For example, we need to confirm whether the minimum concentration of samples is under the human threshold. We tried 4 kind of odorants in this study. By broadening the range of samples, it is possible for us to find more properties of OBP and OR.

Moreover, for the life time experiment of ORs, we can see that the peeling of cells coated on the QCM surfaces happened. Finding a better coating method of cells expressing OR may help to extend the life time of such sensor.

Acknowledgement

I wish to thank Prof. Krishna Persaud of University of Manchester for providing OBPs and telling us their immobilization method. I want to thank Prof. Ryohei Kanzaki, University of Tokyo for providing cells expressing olfactory receptors.

I also wish to thank Mr. Dani for OBP coating, Mr. Sukekawa for cultivating OR, and other members in our lab for teaching me the method of measurement and giving advices to me.

In promoting this research, I received great instruction and numerous advice from my supervising professor, Takamichi Nakamoto. I express my deep appreciation from the bottom of my heart.

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# Appendix

## 1. Usage of FT245RL USB-Parallel Switching module

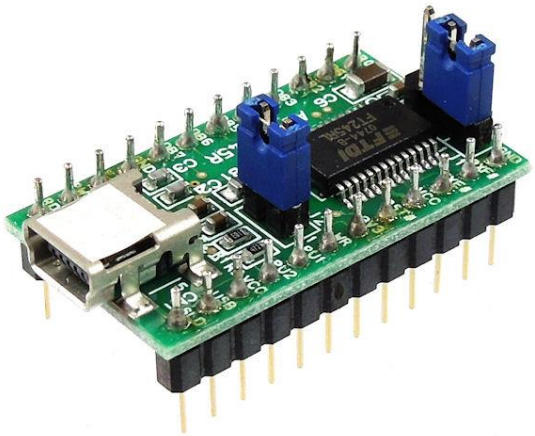


Fig. 1 FT245RL module

Fig. 1 shows the actual thing of FT245RL module. It can realize USB-8bit parallel 2-way data transfer. In this study, we just use the output function. It has 24 pins, 2 jointer and 1 mini USB port. Just using a USB-miniUSB cable can connecting computer with FT245RL module.

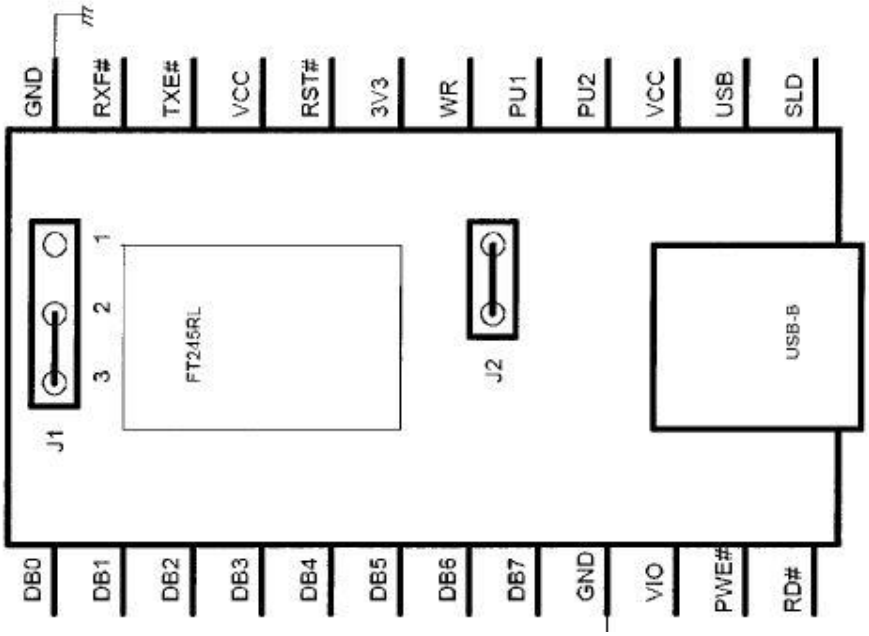



Fig. 2 Pins of FT245RL module

Fig. 2 shows the pins and 2 jointers (J1 and J2) of FT245RL module. DB0 ~DB7 are the I/O pins. In this study, they were used as the output of FT245RL module. To enable the USB power supply, we must let J2 short. And to set the output voltage of the I/O pins to 5V, we must let J1 2-3 short as shown in Fig. 2.

Before using the module, we must download the USB driver from the homepage of FTDI.co. After installing it, the module can be used in Matlab directly. More detailed contents about the module can be seen at http://akizukidenshi.com/catalog/g/gK-01799/.

## 2. The setting method of relative concentration

The relative concentration of sample gas means the proportion of the time that sample gas is floated within a certain range of time. And how the gas is flowed is controlled by the solenoid valve. Therefore, by setting the duration time of the state of solenoid valve, we can control the relative concentration.

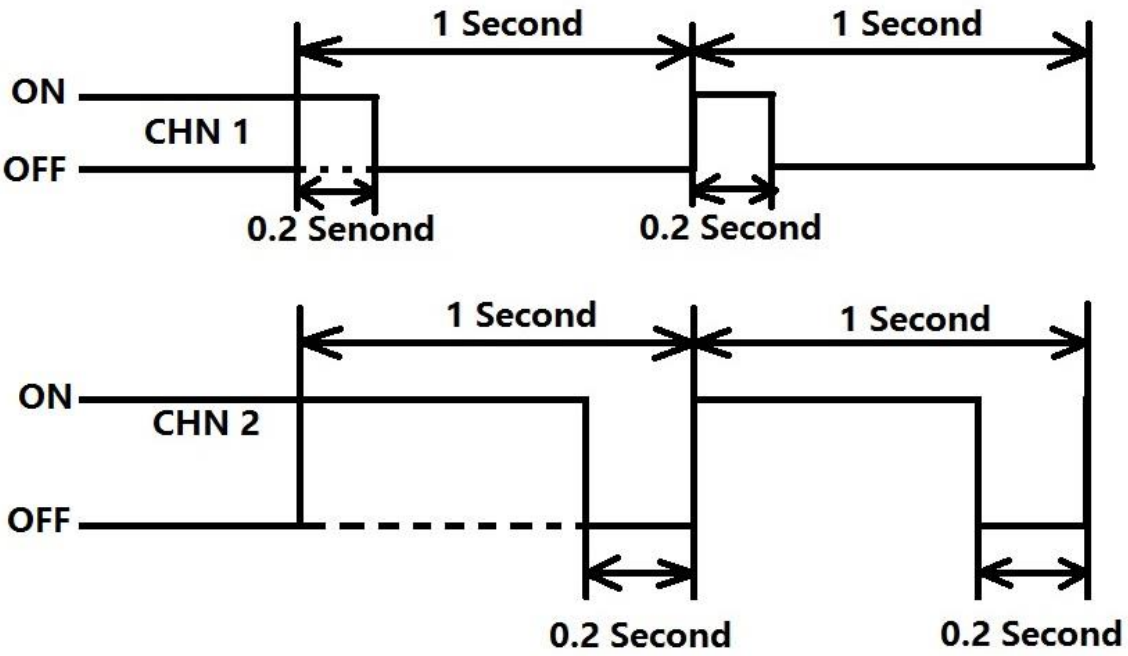



Fig. 3 State of solenoid valve

We take an example in Fig. 3 which shows the relationship between the state of solenoid valve of Channel 1,2 and relative relationship. In Fig. 3, for CHN 1, within 1 second, the state of solenoid valve is on for 0.2 second and for CHN 2, within 1 second, the state of solenoid valve is on for 0.8 second. It means within 1 second, the sample gas is floated for 0.2 second in CHN 1 and 0.8 second in CHN2. Therefore, in Fig. 3, the relative concentrations of CHN 1 and CHN 2 are 20% and 80% individually.

# 3. Measurement program in Matlab

## 3.1 Sending signal to FT245RL

The eight I/O pins of FT245RL module are connected to the eight channels of odor blending system. One I/O pin is responsible for one channel. In the measurement system, if one I/O pin is set as high voltage, the solenoid valve connected to the sample bottle of its corresponded channel is on. For example, if the solenoid valves connected to the sample bottle of CHN2 and CHN5 are set to on, others are set to off, the signal "00010010" is send to FT245RL module from Matlab. Before sending the signal, we need to change binary to decimal. By using the pause() function, we can set the time duration. Pause(x) means waiting x second for execute the next command. The timer() function can also be used to the set the time duration.

One example is shown as follows. The sample first sets the serial communication information and opens the serial communication. Then CHN7 is open for 0.05 second and closed for 0.95 second 60 times. It means the relative concentration is set as 10% every 1 minute within 1 minute. By adding for cycle, changing the parameter of pause(function), changing the sending signal, the relative concentration and time duration can be controlled.

```
s = serial('COM4');
set(s,'BaudRate',9600);
fopen(s)
    for i=1:1:60
```

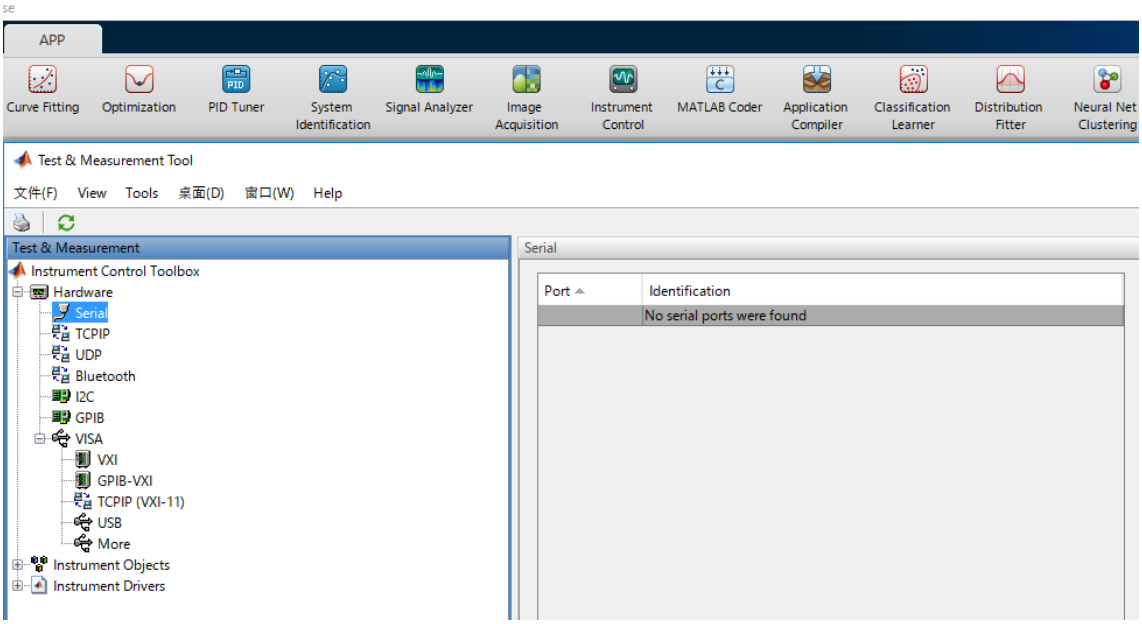
```
        fwrite(s,64)
        pause(0.1)
        fwrite(s,0)
        pause(0.9)
      end
fclose(s)
```

3.2 Reading data from frequency counter by Matlab

The frequency counter used in this study is connected to the computer by serial communication. We have 2 ways to read data from frequency counter by Matlab.

First way is using the app supplied by Matlab.

Push the “APP” button. We will see some app tools in Matlab. We use the “Instrument control”. Open it. The screen is shown as follows. By using the serial function at the left side of the screen, we can realize the communication to the frequency counter.




Second way is to directly input the command.

Two samples are shown as follows. The first sample read the data by serial and write the data into the txt file. The second sample does almost the same thing. But it is written by function.

1st sample:

```
%Name：Matlab Serial
%Description：Read by serial and write to txt file
s=serial('com2');
s.BytesAvailableFcnMode='byte';   % serial setting
s.InputBufferSize=4096;
s.OutputBufferSize=1024;
s.BytesAvailableFcnCount=100;
s.ReadAsyncMode='continuous';
s.Terminator='CR';
fopen(s);                          %open serial port
out=fread(s,7,'uint8');     %read 7 bit one time
fprintf('%3c',out);

fid=fopen('serial_data.txt','w+');     %open txt file
fprintf(fid,'%3c',out);                % write into the txt file
fclose(fid);

fclose(s);
delete(s);
```

2nd sample:

```
%open serial port
function scom = OpenSerial(sname, HReadFcn)
scom = serial(sname);
scom.BytesAvailableFcnMode = 'terminator';
scom.Terminator = '.';
scom.BytesAvailableFcn = HReadFcn;
try
fopen(scom);
catch err
fprintf('%sFail in opening port。\n', sname);
end
fprintf('%sSucceed in opening port。\n', sname);
end
```

```
% write data
function WriteSerial(scom, str)
fprintf(scom, str ,'async');
end
% function of reading data
function ReadFcn_Com2(obj, ~)
n = get(obj, 'BytesAvailable');
if n
a = fread(obj, n, 'uchar');
c = char(a');
fprintf('%s\n', c);
end
end
% close the serial port
function CloseSerial(scom)
try
fclose(scom);
catch err
fprintf('%sFailed in closing port。', scom.Name);
return
end
delete(scom);
end
```

# 4. Print board of solenoid valve controlling board

Fig. 4 shows the print board of solenoid valve controlling board. D1~D32 are the 18V zener diodes. J8~J11 are the sockets connected to the channels of odor blending system. U3 and U4 are the transistor arrays (ULN2083AN). JP4 is the socket for inverter chip (TC74HC540A).

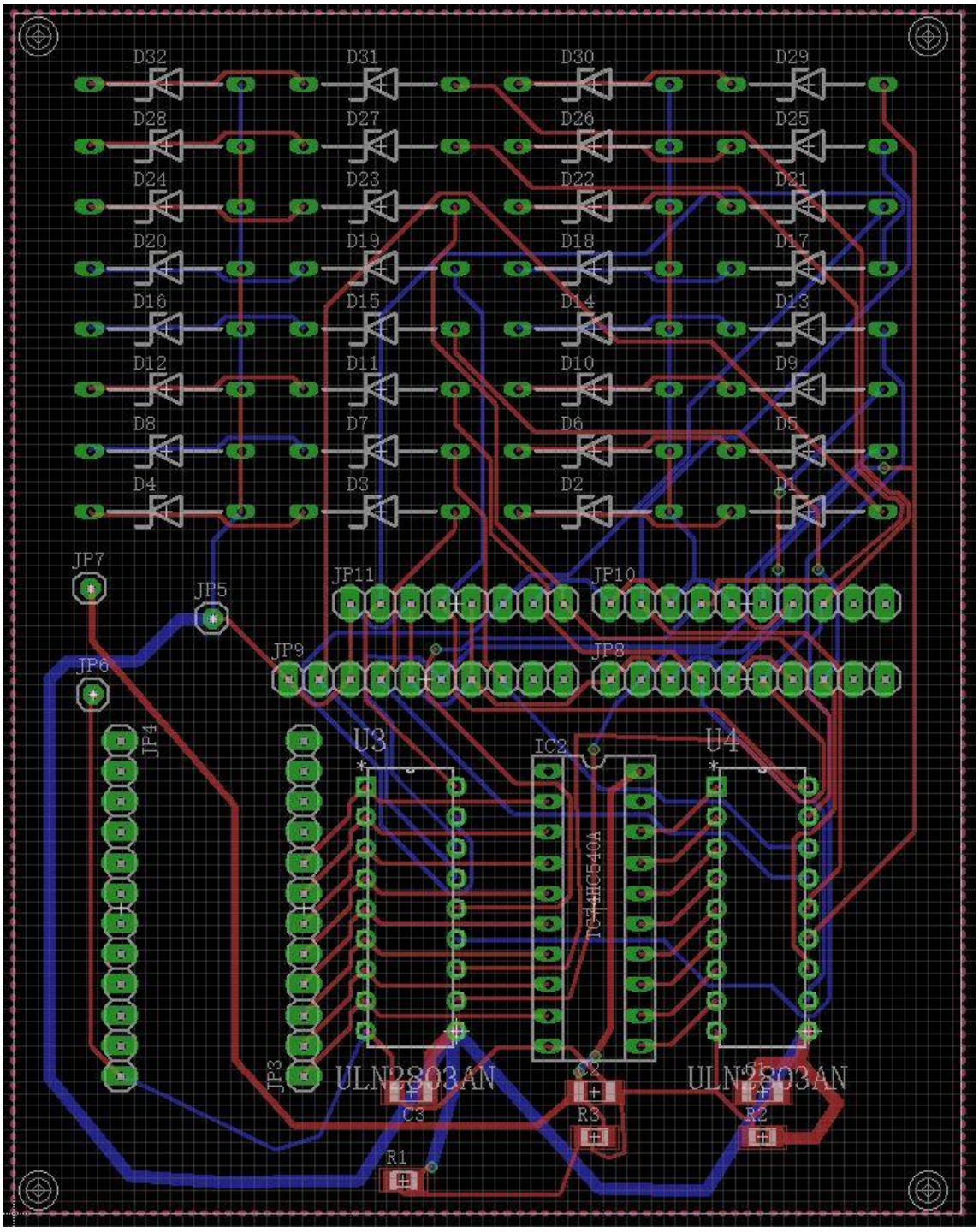



Fig. 4 Print board of solenoid valve controlling board