Recognition of terpenes using molecular imprinted polymer coated quartz crystal microbalance in air phase

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Abstract

Quartz crystal microbalance (QCM) sensor which covered by molecular imprinted polymer (MIP) for terpene was fabricated. The MIP-QCM sensor was designed for enhance the sensor selectivity. The MIP membrane contained the methacrylic acid, which formed specific cavities originated by target molecule. The ingredients for MIP membrane was polymerized on the surface of QCM substrate. The sensing property was examined in gas phase for evaluate the sensor sensitivity and selectivity. The MIP-QCM sensor can detect terpene-contained gas. The sensor characteristics were strongly influenced by the composition ratio of cross-linker, functional monomer and template molecule. The response of the sensor towards terpene gases was partially reversible, however, the terpene gas partially remained in the MIP membrane. The remained molecule can be removed by immersing in a specific solution, and sensor can be used repeatedly. The sensor can distinguish the resemble gas successfully. Additionally, the newly fabricated MIP-QCM sensor showed particularly high sensitivity than that of conventional method. © 2006 Elsevier Ltd. All rights reserved.

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1. Introduction

Terpenes are generic name of oxygenated hydrocarbons is generated by plants. It is helpful for human health, such as human relaxation, deodorant [1] and ozone removal [2]. Therefore, some of terpenes are articles for sale as fragrance. Terpenes show diversified odor despite their resemblance in molecular structure; therefore, the recognition of specific terpene is difficult. On the other hand, the research on electric-nose (E-nose) is getting a lot of attention because it is essential for the development of robots. Therefore, the recognition of terpenes has importance.

Generally, high-performance liquid chromatography (HPLC) [3] or gas chromatography/mass spectrometry (GC/MS) [4,5] can be employed for the quantification of hydrocarbons such as terpenes. However, the sensing device still of a large size and is expensive. In addition, it also takes long time for quantification.

Quartz crystal microbalance (QCMs) was widely researched on application for sensor devices for its high sensitivity, durability and linearity for mass of the target materials. A number of materials have been successfully employed in the detection with QCMs such as toxic gases and tobacco smoke [6–12]. The QCM sensor was also applied for detection of terpenes [13]; however, it can be detected in only liquid phase and not in the gaseous phase.

Molecular imprinted polymer (MIP) was widely researched in several years because it has excellent selective adsorption characteristics. MIP film is yielded by polymerization of the solution containing cross-linkers, functional monomers, and target molecules. The cross-linker was used for forming polymer film. Functional monomer was used for connecting the polymer film to the target monomer. After polymerization, the target molecules were removed and the unique cavity which keeps the form of target molecules was gained. Some group applied the technique to QCM sensor [14–16], however it was not easy. There are two causes. One is the poor connection force between QCM substrate and MIP. Another one is the form of resultant MIP. In most of the reports on MIP,
the MIP was gained as nano-particle. However, the nano-particle is not suitable for QCM type sensor. To overcome these problems, several methods were suggested. Matsuguchi et al. applied the MIP method to gas sensor for VOC gases [17]. In the report, avoid PMMA was used for binding the MIP particle. However, the sensor characteristics dropped down because of the presence of PMMA. The limitation of gas detection was 200 ppm, the gas sensitivity was not enough.

Recently, our group found a simple method for fabricating MIP-coated QCM sensor [18]. The QCM substrate was coated by self-assembly monolayer (SAM) which contains amine functional group. After coating SAM, the precursor of MIP was casted on the QCM and polymerized by heating. By using this process, the MIP can be formed on QCM substrate not as particle but as thin film. The bonding between the amine group and functional monomer of MIP is helpful for forming MIP-QCM. The MIP-QCM type gas sensor was fabricated successfully without a binder such as PMMA.

In this research, The MIP-QCM was examined as a gas sensor for detecting and distinguishing the terpenes. The best suited molar ratio of target molecules was determined after which the sensor characteristics (sensor sensitivity, selectivity and effect of humidity) were examined.

2. Experimental section

2.1. Material

Ethylene glycol dimethacrylate (EGDMA) as a cross-linker, methacrylic acid (MAA) as a functional monomer, 2,2'-azobisisobutyronitrile (AIBN), ethanol, acetic acid as initiator, methanol, α-pinene, limonene, limonene oxide as target terpenes and 2-aminoethanethiol (AET) as SAM were purchased from Wako pure chemicals. All commercial chemicals were used without further purification. Deionized water ($\sim 18 \text{ M} \Omega \text{ cm}$) was used as solvent.

2.2. Target gases

Three terpene materials were used in this research. The molecular structure and properties of these materials are shown in Fig. 1 and Table 1, respectively. The molecular structure of these materials is very similar; however, the smell has large difference. These materials were popularly used for fragrances.

![Molecular structure of target gases](image)

**Fig. 1.** Molecular structure of target gases.

| Property of target gases | Molecular structure | Flavor | Boiling point (°C) | Flash point (°C) |
|-------------------------|---------------------|--------|-------------------|-----------------|
| Limonene C$_{10}$H$_{16}$ | Fresh orange | 165 | 46 |
| Limonene oxide C$_{10}$H$_{16}$O | Green citrus | 113–114 (50 Torr) | 70 |
| α-Pinene C$_{10}$H$_{16}$ | Pine or resinous | 156 | 32 |

2.3. Preparation of QCM sensor

AT-cut quartz crystal, 10 MHz with Au electrode was used as the substrate. The quartz was immersed in solution (the volume ratio is $\text{H}_2\text{O} : \text{IPA} : \text{KOH} = 40:60:1$) for 5 min with ultrasonic action for hydrophilic treatment. After hydrophilic treatment, the QCM substrate was immersed in AET solution for 3 h, and rinsed by immersing in ethanol for 5 min with ultrasonication. The QCM substrate was coated by SAM in this treatment [16].

2.4. Preparation of MIP solution

The terpenes as a template was mixed with the functional monomer MAA, the cross-linker EGDMA and AIBN as a initiator. The solution was deoxygenated by bubbling the nitrogen gas for 10 min. Non-imprinted polymer (NIP) solution which did not contain terpenes was prepared for comparison.

2.5. Preparation of MIP-QCM sensor

The schematic image of the fabrication process of MIP-QCM is shown in Fig. 2. Two microliters of MIP solution was directly cast on the surface of QCM substrate after SAM was coated. The QCM substrate was placed in the oil bath, keeping the temperature at 60 °C. The oil bath was purged by nitrogen gas. The resultant QCM was immersed in extract solution, which contains methanol and acetic acid (15:0.5 wt%) for extract template molecules. The sensors for α-pinene, limonene and limonene oxide were fabricated and named P-MIP, L-MIP and LO-MIP, respectively.

![Schematic image of MIP sensor fabrication process](image)

**Fig. 2.** Schematic image of MIP sensor fabrication process: (a) QCM substrate, (b) coating of SAM, (c) MIP casting and polymerization, (d) extracting process of target molecules.
2.6. Measurement of sensor characteristics

The properties of the gas sensor to terpene gas were measured by the frequency shift response with flow gas system shown in Fig. 3. The N₂ gas was divided into dry gas and wet gas with bubbling, then these gases were used for dilution of the terpene gas. The terpene gas was generated by bolatilizing in temperature keeping bath. The concentration of the gas was controlled by modulating the temperature or adjusting the shape of the beaker which charged terpene solution. Relative humidity of diluted gas was controlled at 50%. The temperature in sensor cell was stabilized at 25 °C.

3. Results and discussion

3.1. Determination of MIP composition ratio

The relationship between the composition ratio of materials and film mass was discussed. Table 2 showed the composition ratio in fabricating MIP (or NIP) film on QCM substrate and mass of MIP film. Film mass was calculated by measuring the resonance frequency of QCM before and after coating the MIP film. By comparing the results of compositions 1 and 2, it was found that the composition ratio of MAA had influence and the loading mass. In polymerization process, connection between MIP and SAM was built on the surface of QCM due to carboxylic group of MAA and amine functional group of AET. Therefore, the amount of MAA has strong influence on the mass of film. In the case of less amount of MAA, large amounts of MIP were peeled by immersing in methanol and acetic acid, it resulted in less mass of film. Similar phenomenon was also observed in comparing compositions 6, 7 and 8. The MIP was formed on the surface of QCM without SAM in the same way. However, the MIP was completely peeled in immersing methanol. The utility of SAM was confirmed by this experiment.

The frequency shift of each sensor exposed to 14.35 ppm of limonene gas is shown in Table 2. The compositions 5 and 7 showed high frequency shift in these samples. In these two samples, the molar ratio of MAA and EGDMA were set at 4 and 20, respectively, and the molar ratio of the target molecule was different. Therefore, the relationship between the quantity of target molecules and frequency shift exposed to limonene gas was examined and the result is shown in Fig. 4. According to this figure, the molar ratio of target molecules had optimal value. In the case of low quantity of target molecules blended, the small quantity of cavities originated by target molecules were formed, which resulted in low frequency shift. On the other hand, when the excessive target molecules were blended in MIP, the cavity could not be formed suitably because the excessive target molecules worked as impurity in polymerization reaction, which resulted in low frequency shift exposed to limonene gas. The best-suited composition ratio of MIP for sensing limonene gas was determined by this experiment.

To discuss the location of the adsorption phenomenon, we investigated the influence on MIP quantities. One microliter of MIP in composition 7 was cast on QCM substrate and the frequency shift exposed to limonene gas was examined. The sensor showed the sensor response to limonene gas, and the resonance frequency shift was 34.6 Hz. The frequency shift was nearly half of that for composition 7 (Table 2), when 2 μL of MIP was cast on QCM substrate. It means that the quantity of cast MIP and the frequency shift exposed to gas has linearity. This result proved that adsorption of MIP-QCM sensors was carried out not only on the surface but also on the inside of MIP.

Fig. 5 showed time-dependent resonance frequency shift of MIP (composition 7) and NIP (composition 1). The sensors

![Fig. 3. Schematic of flow gas system.](image)

![Fig. 4. Frequency shift exposed to limonene gas (14.35 ppm) as a function of blended quantity of target molecules.](image)
were exposed to 14.35 ppm of limonene gas for 10 min, and then were brought back to the nitrogen atmosphere. The frequency shift of MIP and NIP were 64.6 and 12.7 Hz, respectively. The molar ratio of MAA and EGDMA were same in these samples, only the quantity of target molecular was different. Therefore, the result proved that the sensor response was originated by the adsorption of limonene to the cavity of MIP. However, it was considered that a part of sensor response of MIP was not by cavity of MIP but by any other part of MIP because the NIP responded to limonene gas.

3.2. Influence of humidity

The influence of humidity was examined. The L-MIP and NIP was exposed to 14.35 ppm of limonene gas in the humidity at 0 and 50%RH, respectively. The resonance frequency shift is shown in Fig. 6. As is shown in this figure, the sensor sensitivity was decreased in lower humidity. The frequency shift of L-MIP and NIP in 0%RH was 42.7 and 4.2 Hz, respectively. It is considered that the hydrophilicity of MAA influences sensitivity. For determining the concentration of limonene gas, the humidity should be measured in the same time.

3.3. Selectivity

To confirm the sensor selectivity of MIPs, the sensors were exposed to limonene, limonene oxide and pinene gas. The LO-MIP and P-MIP were fabricated by the same process and same composition ratio of MIP solution to L-MIP. The resultants had nearly same film mass to L-MIP.

Fig. 7 shows the selective property of MIP-QCM sensor manufactured by composition 7. According to this figure, the three MIP sensors responded to each gas and the frequency shift was unique. In each MIP sensor, the sensor response was maximized when exposed to target gas. For example, L-MIP showed larger frequency shift than LO-MIP and P-MIP when exposed to limonene gas. It means that the sensor can distinguish the target gas with other similar gases. On the other hand, LO-MIP and P-MIP, which fabricated in same composition ratio as L-MIP, also showed distinguished capability.

The limonene oxide gas showed more unique property than other gases. LO-MIP responded 33.6 Hz, whereas the L-MIP and P-MIP responded 10.1 and 12.8 Hz; in respectively. The difference in the frequency shift was larger than in the case of other gases. In general, the larger difference between the exposed and target gases is due to larger frequency shifts. In the three gases, only the limonene gas has oxygen. It resulted in best distinguishing characteristics of LO-MIP.

It was considered that the response of limonene oxide gas to LO-MIP was attributed to the interaction relating oxygen. Since, the limonene and \( \alpha \)-pinene did not have shapes with similar to that of limonene oxide, there was less interaction with LO-MIP.
In this research, it was confirmed that the sensor for multiple terpene gas can be fabricated in a unified way. This result suggested that the sensor fabrication process and composition ratio can be applied to other terpenes also.

The sensor sensitivity of the newly fabricated MIP-QCM sensor was particularly greater than that of the conventional MIP-QCM. The MIP-QCM of conventional method [17] can detect 200 ppm of VOC gases. On the other hand, the newly fabricated MIP-QCM sensor can detect 10 ppm of the gases. The difference in sensor sensitivities was due to the presence of binder materials. Fig. 8 shows the conventional process for fabricating MIP-QCM sensor. In this process, (a) the precursor was polymerized by heating or irradiation of UV, (b) brough out, (c) murled to particles, (d) cavity was made by immersing to extractive solution and (e) coated on QCM with binder. As a binder, PMMA or PVC was used in conventional reports [17,19]. Using this method, the binder is required because the binding force between the MIP particles and QCM substrate [17,19]. Using this method, the binder is required because the MIP-QCM sensor can detect 10 ppm of the gases. However, the sensor characteristics dropped down because of the presence of PMMA. By the new method (described in Fig. 2), (a–b) QCM substrate was coated by SAM, (c) precursor was casted on QCM substrate directly and polymerized by heating, (d) cavity was made by extractive solution. In this process, the binder material was not required because the MIP was gained in the shape of film. The bonding between the amine group and functional monomer of MIP is helpful for forming MIP-QCM.

4. Conclusion

In this research, the MIP film was successfully applied to gas sensor by using QCM electrode. The sensor sensitivity strongly influenced the composition ratio of cross-linker, functional monomer and the target molecule. In the case of large amount of functional monomer included, the film mass was large. The best composition ratio of cross-linker, functional monomer and target molecule was composition 7 (target:MAA:EGDMA = 1:4:20). The sensors for limonene, limonene oxide and pinene gas can be fabricated in a unified way. The three resembling gases can be distinguished by this method. The sensor can detect the terpene gases of the order of approximately 10 ppm. The sensor sensitivity was particularly increased when compared with the conventional report because the newly suggested method to fabricate MIP does not necessitate a binder such as PMMA.

In applying the MIP-QCM sensor to air phase, there are some typical problems. The first is the influence of humidity. In this study, the sensor sensitivity was increased on increasing in humidity. The relationship between sensitivity and humidity should be determined for measuring accurate concentration. The second one is selectivity. The sensors have selectivity in this research; however, more excellent selectivity is needed for determining the molecular species. For improving this point, it is effective to select suitable cross-linker and functional monomer for each gas. However, this method has potential in gas detection because the method is simple and is widely applicable. It is considered that the gas sensor with excellent selectivity can be fabricated by overcoming these problems.

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