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A Novel Mass-Sensitive Sensor Based on β -Cyclodextrin-Anchored Bisphenol A-Imprinted TiO₂ Ultrathin Layers

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A novel approach to fabricating artificial receptors based on the synergic effect of molecular imprinting and host (β -cyclodextrin)-guest (bisphenol A, BPA) interaction in TiO₂ ultrathin films prepared by the gas-phase surface sol-gel process was reported. Quartz crystal microbalance (QCM) electrodes were used to confirm the mass-sensitive change due to BPA incorporation and guest binding into the film with the aim of developing a BPA sensor. The concentration of 1 μ M (272 ppb) BPA was successfully measured and the limit of detection was estimated to be 0.1 μ M (27.2 ppb) BPA. The affinity of the imprinted film to BPA, which was calculated using a Benesi-Hildebrand plot, was ca. 8 times higher than that of the nonimprinted film. This selective binding feature in principle can be applied to more complex organic compounds.

1. Introduction

Molecular imprinting is a representative technique for the fabrication of artificial receptors, which can be applied to sensing, catalysis and separation materials.^(1,2) The advantages of molecularly imprinted materials over biological receptors include their mechanical and chemical stability, low preparation cost and wide range of operating conditions. Organic polymers that are widely used for molecular imprinting, in spite of their sensitivity and selectivity, possess some limitations that make their practical application in sensors rather complicated.⁽³⁾ In our previous works, we have demonstrated that molecularly imprinted inorganic TiO₂ ultrathin films prepared by the surface sol-gel process can be successfully used as receptors with high sensitivity and selectivity to target analytes.^(4,5) However, this approach is not fully satisfactory for all chemical compounds because of the limited binding affinity of TiO₂ gel matrices.

One alternative method of improving the binding capability of TiO₂ gel matrices is to demonstrate additional chemical interactions. Recently, some useful challenges to

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obtain environmentally robust and selective binding sites by a combination of host-guest interaction and molecular imprinting have been reported.⁽⁶⁾ Among host compounds, cyclodextrins (CDs) can be a useful candidate for this purpose because they have apolar cavities with different molecular sizes of 5–8 Å in interior diameter and 7 Å in depth and can be applied to hydrophobic interaction.⁽⁷⁾

On the other hand, the quartz crystal microbalance (QCM) device is extremely sensitive and a powerful tool for monitoring mass changes in the nanogram range.^(8,9) The basic principle of QCM sensors is the measurement of the frequency shift as a result of the mass adsorbed on the QCM resonator. How to modify the surface property of the quartz resonator becomes an essential issue for enhancing the range of applications of QCM sensors.⁽¹⁰⁾

In this work, we propose a novel imprinting approach using the inclusion capability of CDs in TiO₂ ultrathin films alternately deposited by the gas-phase surface sol-gel (GSSG) process onto QCM electrodes. The advantage of the proposed method is the preservation of the specific complex structure between CD and template molecules because the TiO₂ gel imprinting matrix can be prepared from a gas-phase metal alkoxide precursor without organic solvents that are involved in a conventional sol-gel process. Bisphenol A (BPA) was chosen as a template molecule because its detection is important for environmental quality analysis^(11–13) and the investigation of biological damage,⁽¹⁴⁾ and has become an index for sensing structurally related harmful substances.

2. Experimental

2.1 Materials

Ti(O-*n*Bu)₄ (Mw: 340.34) was purchased from Kishida Chem. β -Cyclodextrin (β -CD, Mw: 1134.98), bisphenol A (BPA, Mw: 228.29), diethylstilbestrol (DES, Mw: 268.36), and β -estradiol (β -ED, Mw: 272.39) were purchased from Tokyo Kasei Chem., Japan. Dienestrol (DS, Mw: 266.3), genistein (GS, Mw: 270.24) and 4-(*tert*-octyl) phenol (TOP, Mw: 206.32) were purchased from Sigma-Aldrich. Hexestrol (HS, Mw: 270.38) was purchased from Wako Pure Chem., Japan. Deionized water (18.3 M Ω ·cm) was obtained by reverse osmosis followed by ion exchange and filtration (Nanopure Diamond, Barnstead, Japan). All these chemicals were guaranteed reagents, and used without further purification.

2.2 Preparation of imprinted TiO₂ films

The alternate deposition of TiO₂ gel and CDs was described in our previous work.⁽⁶⁾ Briefly, two steps are involved and a schematic illustration of the method is shown in Fig. 1. First, titanium *n*-butoxide, Ti(O-*n*Bu)₄, was heated in a sealed bottle at 85°C, vaporized with N₂ gas at a flow rate of 3 L/min and deposited on a mercaptoethanol-modified gold-coated QCM electrode (9 MHz, 0.159 cm² for one side, USI System, Fukuoka, Japan) for 10 min, followed by N₂ gas flushing to remove physisorbed alkoxides. Then the QCM electrode was immersed in deionized water for 1 min and dried with N₂ gas. Subsequently, the TiO₂ gel-deposited QCM electrode was immersed in an aqueous solution of β -CD (10 mM; nonimprinted) or 2:1 β -CD/BPA complex (10:5, mM/mM;

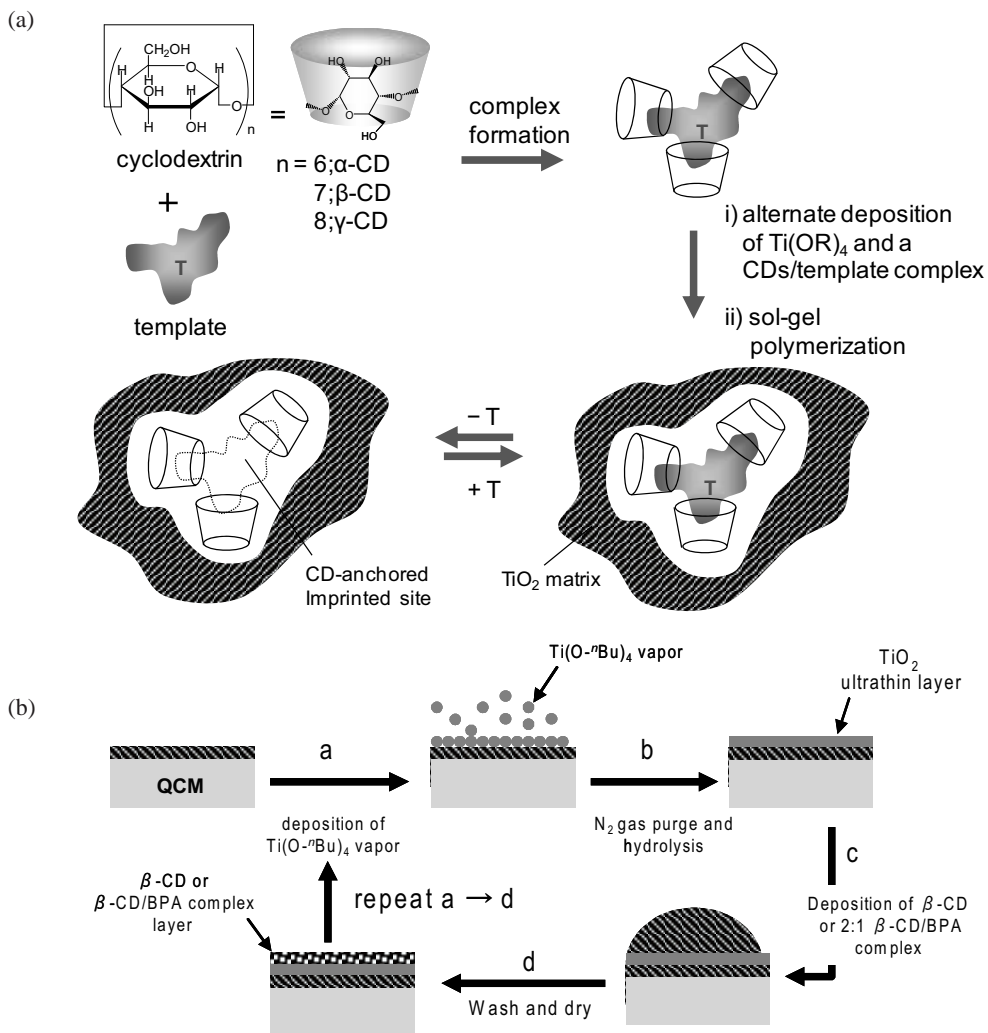


Fig. 1. Schematic illustration of (a) the CD-anchored molecular imprinting and (b) the deposition process of a TiO_2 gel and a $\beta\text{-CD/BPA}$ complex via the gas-phase surface sol-gel process.

imprinted) for 20 min at 30°C , rinsed with deionized water and dried with N_2 gas. This alternate deposition process was repeated 10 times. After each deposition cycle, the frequency was measured.

2.3 Imprinting effect measurement

In order to prepare BPA binding sites, the introduced BPA template was removed by immersing the QCM electrode modified with a $\beta\text{-CD/BPA}$ complex TiO_2 film into

ethanol at 25°C for different time intervals, followed by rinsing with deionized water and drying under N₂ gas. Rebinding was conducted by immersing the BPA-imprinted QCM electrode in 20 μM (5.4 ppm) BPA aqueous solution for different time intervals in order to measure the saturation rebinding time of BPA. After each procedure, the frequency change was measured. For reference measurements, the same experimental procedures were applied to the QCM electrode modified with a nonimprinted TiO₂ film.

The concentration dependence of the sensor response was measured by immersing the BPA-imprinted QCM electrode in BPA aqueous solution of different concentrations for 5 min at 25°C.

3. Results and Discussion

3.1 Deposition of β-CD/BPA complex on TiO₂ gel layer

In our system, a frequency decrease of 1 Hz corresponds to a mass increase of ca. 0.9 ng. The thickness (*d*) of an adsorbed film on one side of a resonator is given by the Sauerbrey equation⁽¹⁵⁾

$$2d (\text{Å}) = -\frac{\Delta F (\text{Hz})}{1.832\rho (\text{g/cm}^3)}, \quad (1)$$

where ρ is the film density and ΔF is the frequency shift of the QCM.

Figure 2 shows QCM frequency shifts due to the alternate adsorption of β-CD or 2:1 β-CD/BPA complex with Ti(O-*n*Bu)₄. The QCM frequency linearly decreased with an increase in the number of deposition cycles in both cases, indicating regular film growth.

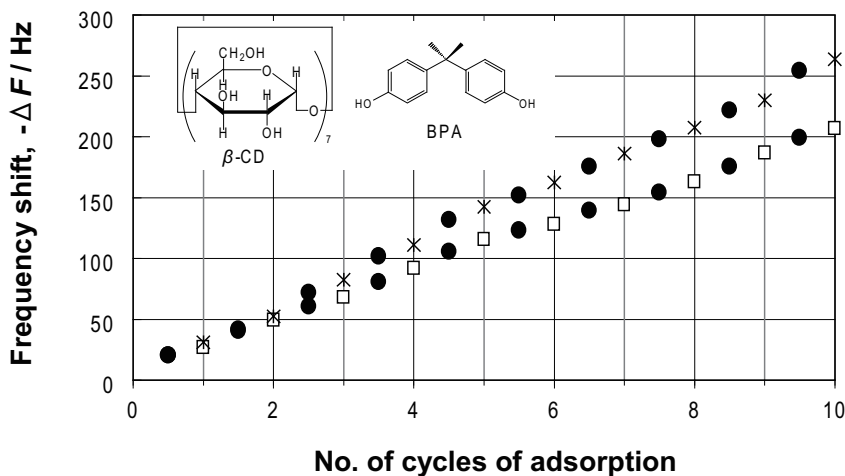


Fig. 2. QCM frequency shift due to the alternate adsorption of β-CD or a 2:1 β-CD/BPA complex with Ti(O-*n*Bu)₄; circles, TiO₂ layer; squares, β-CD; and crosses, β-CD/BPA complex.

The average frequency shifts were 11 ± 2 and 18 ± 7 Hz for $\text{Ti}(\text{O}-i\text{Bu})_4$ and 9 ± 2 and 13 ± 2 Hz for β -CD and the 2:1 β -CD/BPA complex, respectively. The amount of introduced 2:1 β -CD/BPA complexes per layer can be estimated from the average QCM frequency shift, as mentioned in eq. (1).⁽¹⁵⁾ The adsorption density of the complex was calculated from the adsorbed mass corrected by the molecular weight (Mw: 2498.3) of the 2:1 β -CD/BPA complex and the surface area of the QCM electrode for both sides (0.32 cm^2). The 2:1 β -CD/BPA complex film shows an adsorption density of $0.09 \text{ complexes/nm}^2$, and thus, the 2:1 β -CD/BPA complex occupies an adsorption area of $11.2 \text{ nm}^2/\text{complex}$. Thus, the adsorption density for β -CD (Mw: 1135.0) can be estimated to be $0.18 \text{ molecules/nm}^2$. Similarly, the adsorption density of β -CD in the TiO_2/β -CD film was calculated to be $0.13 \text{ molecules/nm}^2$, showing an adsorption area of $7.5 \text{ nm}^2/\text{complex}$. This means that β -CD is effectively employed owing to the presence of BPA and therefore the 2:1 β -CD/BPA complex occupies a larger adsorption area than the β -CD compound without BPA.

3.2 BPA response and imprinting effect

The removal of the BPA template from the film was achieved by immersing the QCM electrode in ethanol at 25°C for different time intervals in order to measure the removal time of BPA, followed by rinsing with deionized water and drying under N_2 gas. Figure 3(a) shows the frequency change due to the removal from and rebinding to the 2:1 β -CD/BPA complex film of BPA. The complete removal of BPA was achieved within 1 min and the frequency change by the desorption process was 12 Hz. The desorption ratio to the total frequency shift (130 Hz) of the 2:1 β -CD/BPA complex was about 9.2%. This figure agrees with the mass composition of BPA in the 2:1 β -CD/BPA complex. Therefore, we conclude that the BPA template was successfully removed.

Rebinding was conducted by immersing the BPA-imprinted QCM electrode into a $20 \mu\text{M}$ BPA aqueous solution for different time intervals in order to measure the rebinding time of BPA. The BPA adsorption was very fast with a saturation time within 5 min. As shown in Fig. 3(a), the frequency changes were the same for both the removal and rebinding of BPA (12 Hz), indicating an almost 100% rebinding efficiency. The concentration dependences of the BPA binding in the imprinted and nonimprinted films were compared (Fig. 3(b)). In both cases, the BPA binding is dependent on the Langmuir adsorption isotherm. However, the BPA-imprinted TiO_2/β -CD film has a higher binding capacity for BPA than the nonimprinted one. The limit of detection estimated using the results obtained from Fig. 3(b) was $0.1 \mu\text{M}$ (27.2 ppb).⁽¹⁶⁾

According to the Benesi-Hildebrand method,⁽¹⁷⁾ the binding constant (K_a) was calculated to be 1.26×10^6 and $1.65 \times 10^5 \text{ M}^{-1}$ for the imprinted and nonimprinted films, respectively. The affinity of the imprinted film to BPA is about 8 times higher than that of the nonimprinted film. The obtained values are one order of magnitude larger than the reported results for β -CD.^(18,19) This indicates the high selectivity of the BPA-imprinted TiO_2/β -CD film to the template compound, which is due to the synergic effect of molecular imprinting and the presence of β -cyclodextrin hosts in TiO_2 ultrathin films. To compare the selectivity of the imprinted and nonimprinted films, guest binding measurements were conducted using different organic compounds structurally similar to BPA. Figure 4 shows the amount of bound molecules calculated from the frequency

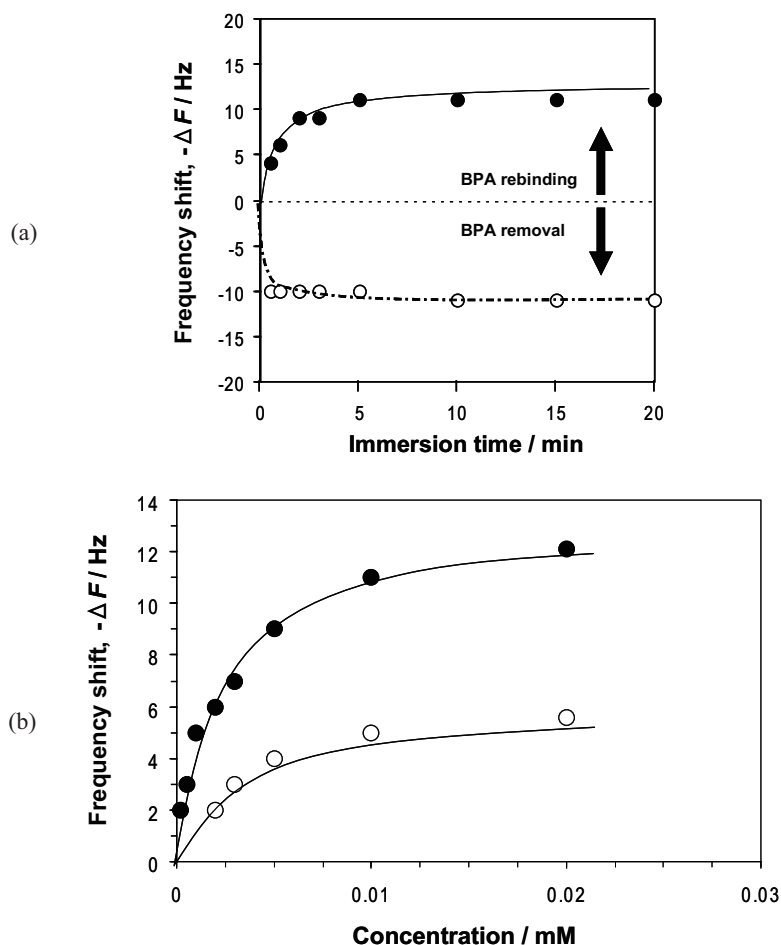


Fig. 3. (a) Frequency shifts due to the removal from (○) and rebinding to (●) the 2:1 β -CD/BPA complex TiO_2 film of BPA and (b) concentration dependences of BPA binding in the imprinted (●) and nonimprinted (○) films.

change after the QCM electrodes were immersed in 20 μM aqueous sample solution. The nonimprinted $\text{TiO}_2/\beta\text{-CD}$ film showed a higher affinity to TOP and about a twofold lower response to BPA than the BPA-imprinted $\text{TiO}_2/\beta\text{-CD}$ film. From these results, it seems that the guest binding of the imprinted film is dependent on the Mw (size) of the guest molecules. For instance, TOP molecules smaller than BPA molecules show easier penetration into the imprinted film than larger molecules. However, the highest binding efficiency is achieved with BPA, indicating the high affinity in the host-guest interaction of BPA between the imprinted $\beta\text{-CD}$ s. In particular, the binding of the relatively large HS and $\beta\text{-ED}$ molecules is suppressed by the current imprinting process; however, these molecules show comparatively better binding to the nonimprinted film.

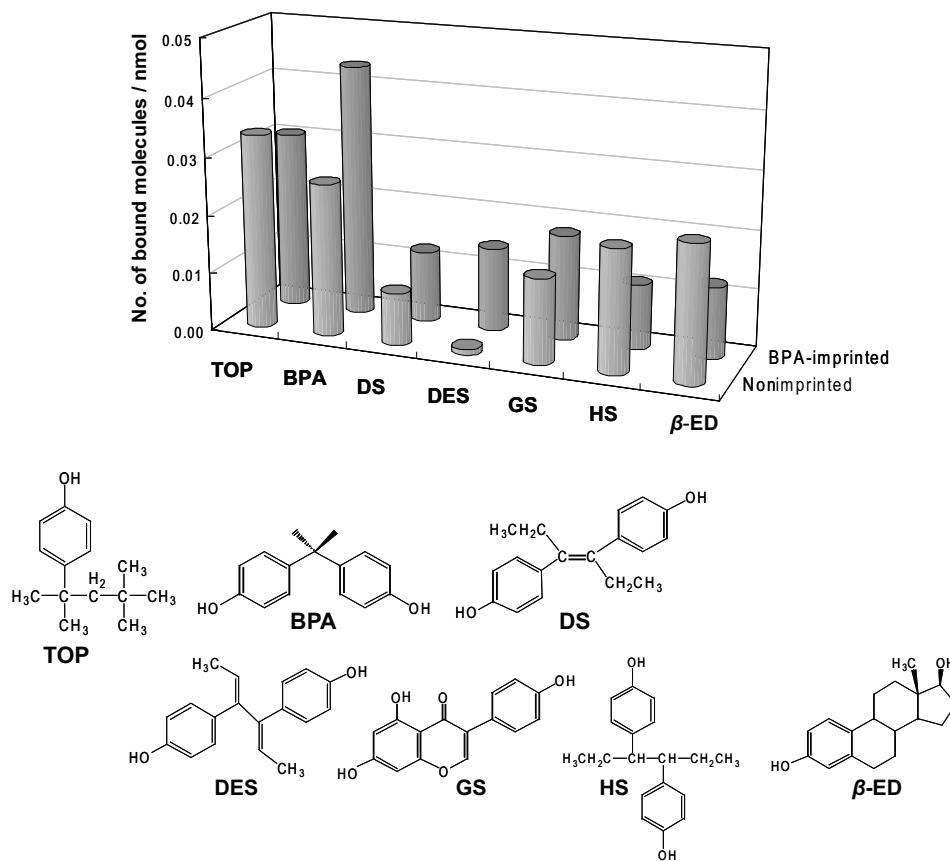


Fig. 4. Guest selectivities of the nonimprinted and BPA-imprinted $\text{TiO}_2/\beta\text{-CD}$ films to several organic compounds: TOP, 4-(*tert*-octyl) phenol; DS, dienestrol; DES, diethylstilbestrol; GS, genistein; HS, hexestrol; and $\beta\text{-ED}$, β -estradiol.

4. Conclusions

In conclusion, the combination of host-guest interaction and molecular imprinting in TiO_2 ultrathin layers was employed to fabricate environmentally robust receptor sites. The gas-phase sol-gel process was used successfully employing a complex of $\beta\text{-CD}$ s and a template inserted into TiO_2 gel layers. Highly sensitive detection of BPA in water was achieved with a limit of detection of ca. 27.2 ppb. The binding constant ($1.26 \times 10^6 \text{ M}^{-1}$) of the BPA-imprinted $\text{TiO}_2/\beta\text{-CD}$ film was 10 times higher than the reported result ($1.3 \times 10^5 \text{ M}^{-1}$)⁽¹⁷⁾ and the selectivity of the imprinted film to BPA was much higher than that of structurally similar organic compounds. The current approach would be extended to more complex organic compounds because cyclodextrin hosts and TiO_2 matrices can be successfully combined for the precise recognition of complex molecular structures.

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