

Facile Fabrication of Molecularly Imprinted Cavities in Spin-coated TiO₂ Nanofilms

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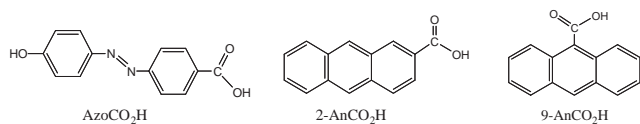
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Molecularly imprinted TiO₂ nanofilms were prepared by spin coating, and the influence of template/matrix precursor ratio was examined. The imprinted films including 10 to 40 times matrix precursor, Ti(O-ⁿBu)₄, provided efficient and selective binding as compared to those of low matrix precursor ratios. The highest binding and selectivity were observed by a TiO₂ film imprinted with 5 mM AzoCO₂H and 100 mM Ti(O-ⁿBu)₄, with template selectivities of 1.6 and 2.1 against 2-AnCO₂H and 9-AnCO₂H-imprinted films, respectively.

Molecular imprinting is a representative technique to memorize shapes and functionalities of given molecules in appropriate solid matrices. Today, this field has become dominated by the use of organic polymeric materials and shows a wide range of applications such as chromatographic separation, enzyme-mimicking catalysts, chemical sensors, and biosensors.¹ On the other hand, inorganic matrices have received much less attention for molecular imprinting, in spite of the fact that they have some advantages such as structural rigidity and high surface area relative to organic matrices.²

Over the last several years, we have studied molecular imprinting for the recognition of organic molecules in ultrathin TiO₂ gel multilayers that were fabricated by the surface sol-gel process.³ Important molecular selectivities such as functional selectivity, structural selectivity, enantioselectivity, and regioselectivity⁴ were realized, and the usefulness of the TiO₂ gel matrix for molecular imprinting has become apparent.⁵ However, its effectiveness as a practical technique has not been fully examined. In this study, we examine two important factors that are considered essential in practical applications. One is to find optimal fabrication conditions for improved selectivity and binding. The other is to develop more efficient film fabrication methods other than the surface sol-gel method.

We used in this study three template molecules that possessed closely related molecular sizes (ca. 7 to 9 Å) and structures (Scheme 1). First, Ti(O-ⁿBu)₄ as matrix precursor was mixed with a given carboxylic acid in toluene/ethanol (1/1, v/v) and then stirred for more than 6 h to form a complex of Ti(O-ⁿBu)₃-carboxylate. The template concentration in the mixture was varied from 0 mM (nonimprinted) to 20 mM against 100 mM Ti(O-ⁿBu)₄. Films were prepared by spin coating (1 min, 4000 rpm) of 10 μL of these mixtures on quartz or glass plates treated with 1 wt % ethanolic KOH (ethanol/water = 3:2,



Scheme 1.

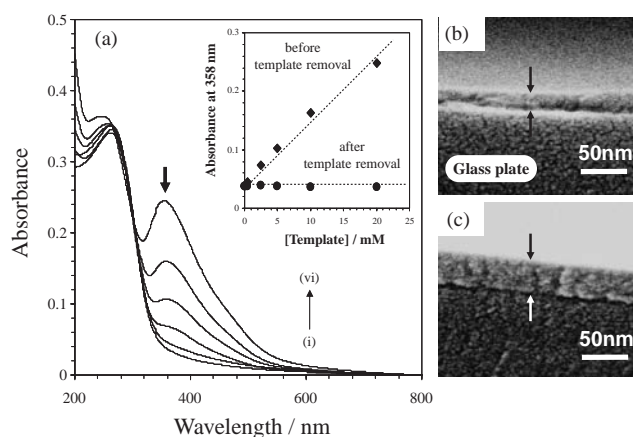


Figure 1. UV-vis absorption spectral changes of AzoCO₂H-TiO₂ gel films (a): Stock solution: Ti(O-ⁿBu)₄; 100 mM, AzoCO₂H; (i) 0, (ii) 0.5, (iii) 2.5, (iv) 5, (v) 10, and (vi) 20 mM. Scanning electron micrographs of the cross section of 0 and 20 mM AzoCO₂H-TiO₂ gel films deposited on glass plates (b and c, respectively). Insert shows the absorbance change at 358 nm before and after template removal.

v/v), and allowed to dry overnight in a humidity chamber (90% humidity at 40 °C).

Incorporation of the template molecules and their removal can be confirmed by UV-vis absorption spectra of the azobenzene and anthracene moieties. Figure 1a shows UV-vis absorption spectral changes, and the absorbance at 358 nm regularly increased in proportion to the concentration of AzoCO₂H in the mixed precursor solution ($\Delta\text{Abs} = 0.01/\text{mM}$, $r^2 = 0.989$). Similar results were obtained with the 2-AnCO₂H- and 9-AnCO₂H-TiO₂ gel films that have maximum absorbances at 261 and 258 nm, respectively, data not shown. The templates incorporated in the TiO₂ matrix were readily removed by treatment with aqueous ammonia (0.1%, v/v). In the case of the AzoCO₂H template, all the films show the absorption of TiO₂ gel film alone after template removal, as shown in the inset of Figure 1a for the UV absorbance at 358 nm. This means that the amount of the TiO₂ gel becomes equal after template removal, irrespective of the template concentration in the original film. The film thickness is naturally enhanced with increasing amounts of template in the precursor solution. Figures 1b and 1c show scanning electron micrographs of the cross section of the 0 and 20 mM AzoCO₂H-TiO₂ gel films deposited on glass plates, respectively. These films are smooth over large areas, and possess different thicknesses of 24 ± 2 and 34 ± 5 nm, corresponding to the amount of template.

The imprinted films were incubated in a solution of 5 mM guest molecules in ethanol for 60 min at room temperature,

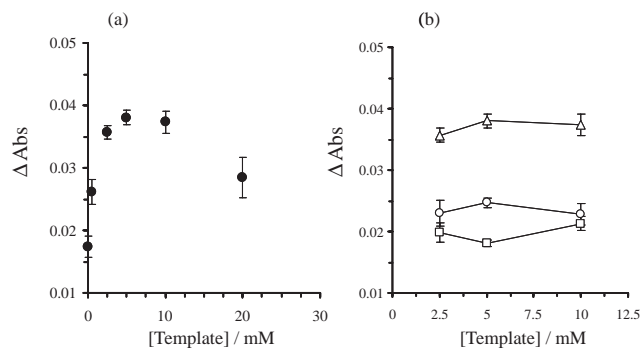


Figure 2. UV absorbance increases at 358 nm due to rebinding of AzoCO₂H to the 0 to 20 mM AzoCO₂H-imprinted films (a). Comparison of AzoCO₂H binding to the (Δ) AzoCO₂H-, (\circ) 2-AnCO₂H-, and (\square) 9-AnCO₂H-imprinted films (template concentration: 2.5, 5, and 10 mM, respectively). Binding conditions: 60 min at rt, in 5 mM AzoCO₂H solution in ethanol ($n = 5$).

rinsed with ethanol, and dried with nitrogen gas. The amount of bound guest molecules can be represented by their absorbance increases. The UV absorbance increase upon guest binding ($\Delta\text{Abs}_{\text{binding}}$) was observed at 258, 261, and 358 nm for 9-AnCO₂H, 2-AnCO₂H, and AzoCO₂H, respectively. The template/TiO₂ ratio of the imprinted film affects the rebinding capability, as shown in Figure 2a. The absorbance increase due to rebinding of AzoCO₂H was maximal for the imprinted films with 2.5 to 10 mM AzoCO₂H. The maximum increase observed with the 5 mM-templated film, corresponded to 58% of the initial absorbance difference ($\Delta\text{Abs}_{\text{initial}}$) before and after template removal: $\Delta\text{Abs}_{\text{initial}}$, 0.065 ± 0.002 and $\Delta\text{Abs}_{\text{binding}}$, 0.038 ± 0.001 . This shows that 58% of the templated cavity is filled with the rebound template molecule. The amount of the rebound AzoCO₂H on this film is about 2 times larger than that on/in the corresponding nonimprinted TiO₂ film (0.038 ± 0.001 vs. 0.017 ± 0.002). Perhaps, the binding of the non-imprinted film is associated with nonspecific Ti–OH residues due to the hydrolysis of the butoxide groups.

The adsorption of AzoCO₂H was also studied for two series of the reference film: 2-AnCO₂H- and 9-AnCO₂H-imprinted films (2.5, 5, and 10 mM films, respectively). As can be seen from Figure 2b, the binding of AzoCO₂H on all the reference films was much less than that on the AzoCO₂H-imprinted films. Guest selectivity of the AzoCO₂H-imprinted films for three template concentrations is estimated to be 1.5, 1.6, and 1.6 against the 2-AnCO₂H-imprinted films, respectively, and 1.8, 2.1, and 1.8 against the 9-AnCO₂H-imprinted films. In both cases, somewhat better binding is observed for the 5 mM AzoCO₂H-imprinted film. Apparently, the TiO₂ film imprinted with 5 mM AzoCO₂H gave highest binding and selectivity.

From these results, we can conclude that the binding efficiency and selectivity of imprinted sites depend on the molar ratio of the template and matrix precursor. In fact, we observed here better binding and selectivity than those described in our previous study.⁴ It appears that 10 to 40-times Ti(O-^{*n*}Bu)₄ is needed for one template molecule in order to construct optimized receptor cavities, where a thin titanium oxide network of single or double layers surround the template molecule (Figure 3b). Interestingly, the binding efficiency and selectivity is depressed, when the template concentration is too low or too

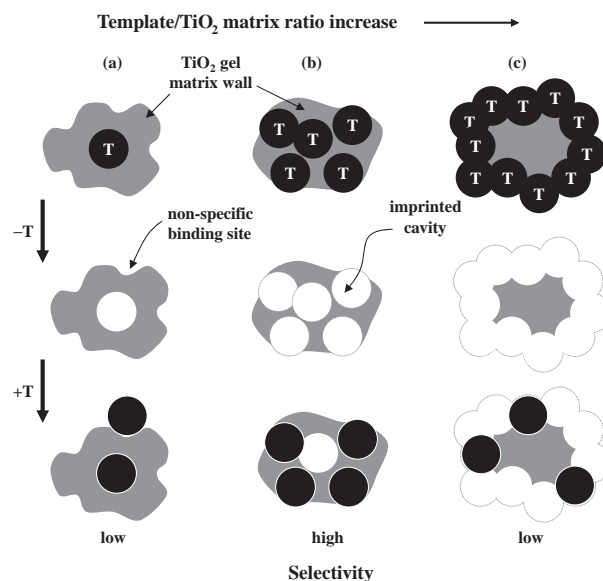


Figure 3. Schematic illustration of TiO₂ gel imprinted cavities. The matrix precursor/template ratio is more than 40 (a), 10 to 40 (b), and less than 10 (c).

high. As illustrated in Figures 3a and 3c for these cases, the guest binding may occur at nonspecific binding sites of the outer surface or the inner pore with lower quality. At higher template concentrations, most of the binding sites would exist on the surface of TiO₂ gel matrices and they can recognize only partial structures of the template, giving lower selectivity. Oppositely, at lower template concentrations, the binding sites may be composed of specific and nonspecific ones, and their selectivity is suppressed. We believe that the current imprinting process is simple and useful to prepare discriminative receptor cavities by controlling the molar ratio of template/matrix precursor. This spin-coating process is less time-consuming, and it becomes possible to examine wider ranges of fabrication conditions.

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