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Tailored Chemosensors for Chloroaromatic Acids Using Molecular Imprinted TiO₂ Thin Films on Ion-Sensitive Field-Effect Transistors

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The SiO₂ gate of an ion-sensitive field-effect transistor, (ISFET), is functionalized with a TiO₂ film that includes imprinted molecular sites for 4-chlorophenoxy acetic acid, (1), or 2,4-dichlorophenoxy acetic acid, (2). The functionalized devices that include the imprinted interfaces reveal an impressive selectivity in the sensing of the imprinted substrates Na⁺-1 or Na⁺-2. The detection limit for Na⁺-1 is $(5 \pm 2) \times 10^{-4}$ M, which corresponds to 38 mV·dec⁻¹ in the concentration range of 0.5 to 6 mM. The detection limit for the analysis of Na⁺-2 is $(1.0 \pm 0.2) \times 10^{-5}$ M, which corresponds to 28 mV·dec⁻¹ in the concentration range 0.1−9.0 mM. The equilibration time of the devices is ca. 5 min.

Molecular imprinted organic polymers or SiO₂ polymer composites[1,2] find broad applications as separation matrixes in chromatography,[3,4] catalytic media mimicking enzymes,[5,6] and selective binding interfaces for sensoric applications.[7] Molecular imprint of recognition sites of carboxylic acid-functionalized substrates in TiO₂ sol−gel-deposited thin films has been developed as a means to tailor specific binding sites in pure inorganic deposits.[8] Imprinted TiO₂ thin films organized on a piezoelectric crystal enabled the microgravimetric quartz crystal microbalance analysis of short peptides and other carboxylic acids.[9] The functionalization of field-effect transistors, (FET), with recognition sites for ions[10,11] or molecular guest substrates[12] or with catalytic units that alter the pH at the FET gate interface[13] were developed as chemosensors. The chemical recognition features introduce the specificity and selectivity into the sensor, whereas the FET provides the sensitivity property of the integrated chemosensor devices. Chloroaromatic acid and, specifically, 4-chlorophenoxy acetic acid (**1**) or 2,4-dichlorophenoxy acetic acid (**2**) are used as common herbicides, and their detection in water reservoirs, soil, or food products is important for monitoring and eliminating environmental pollution.[14] Several biosensors[15] were suggested to detect these herbicides. Here we report on the organization of imprinted TiO₂ thin film-functionalized ion-sensitive field-effect transistors,

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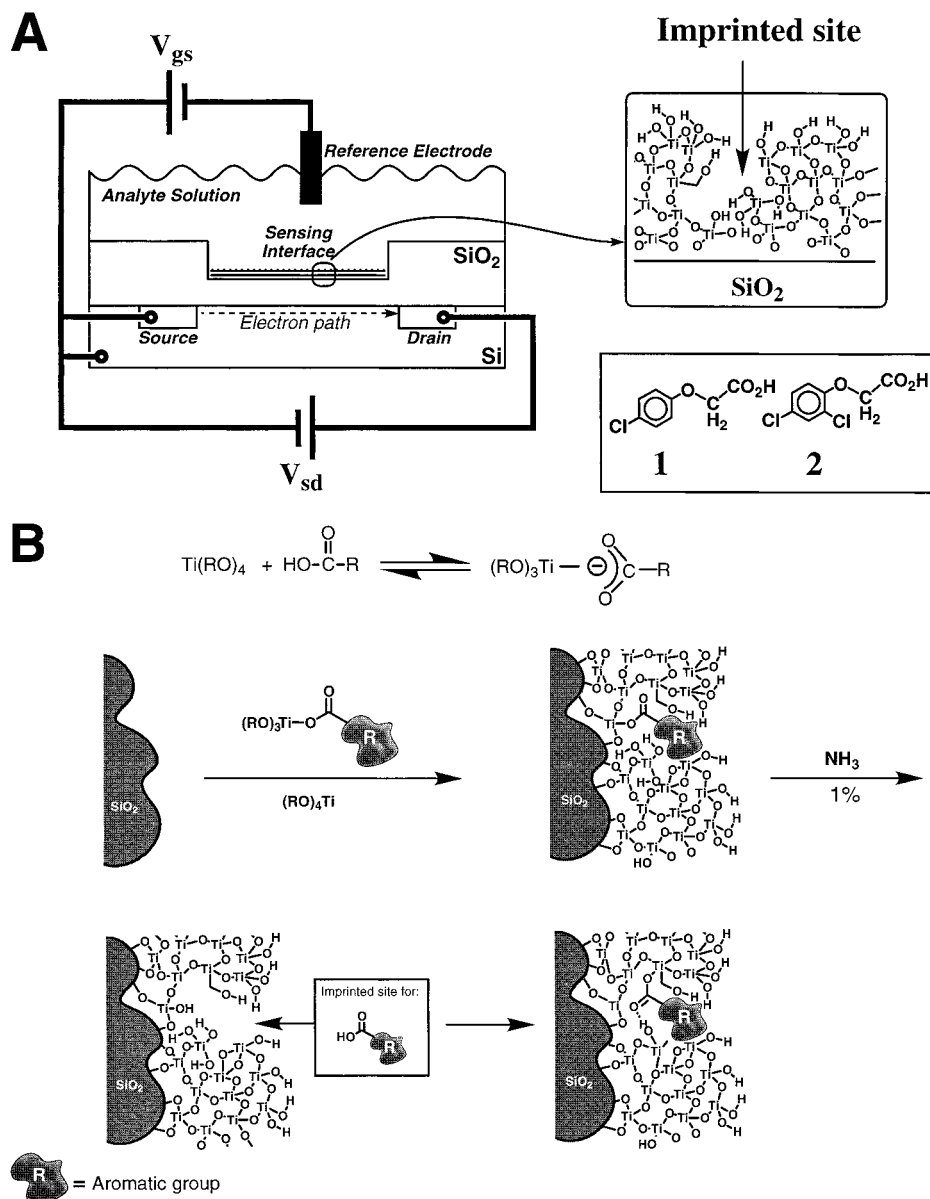
Figure 1. (A) Schematic configuration of the molecular-imprinted FET device. (B) Preparation of molecular-imprinted sites for chloroaromatic acids in a TiO_2 thin film acting as the sensing interface on the ISFET gate.

(ISFETs), for the specific sensing of **1** or **2**.

Figure 1A outlines the configuration of the FET device[16] that includes the ion-sensitive interface that is formed by the deposition of the molecular-imprinted TiO_2 film on the SiO_2 gate. Figure 1B shows the preparation of the chemosensor for chloroaromatic acetic acids. A titanium(IV) butoxide solution (ethanol: toluene, 1:1) was reacted with the respective carboxylic acid, and the resulting mixture that included the titanium(IV) butoxide—carboxylate complex was deposited onto the ISFET gate. The sol—gel polymerization of the mixture on the SiO_2 gate interface resulted in the TiO_2 film with the embedded carboxylate. Treatment of the film with ammonia solution, 1% (v/v), resulted in the elimination of the carboxylate and the formation of imprinted molecular sites for the respective acid in the TiO_2 film.

Figure 2, curve a, shows the changes in the gate-source potential, V_{gs}, of the ISFET device that includes **1**-imprinted interface, at variable concentrations of the Na^+ salt of **1**. The lower limit of detection of **1** is $(5 \pm 2) \times 10^{-4}$ M and the sensitivity corresponds to 38 mV·dec^{-1} in the concentration range of 0.5 to 6 mM. The equilibration time of the device for the sensing of Na^+-**1** is ca. $\tau_{95\%} = 5$ min. The sensing device is reusable after its treatment with an aqueous ammonia solution, 1% (v/v), followed by rinsing of the device with a phosphate buffer solution to regenerate the sensing interface (Figure 2 inset). The slight decrease in the ISFET response upon the cyclic analysis of Na^+-**1** may be attributed to the incomplete removal of the analyte Na^+-**1** that was sensed in previous cycles. A TiO_2 film that lacks imprinted recognition sites for **1** (nonimprinted film) was similarly deposited on the gate interface by the sol—gel polymerization of a

as the drain current (I_d) and the source-drain voltage (V_{sd}) remain constant ($I_d = 100$ μA and $V_{sd} = 1$ V, respectively). The experiments were carried out at room temperature in phosphate buffer solution (pH = 7.2) in the presence of 0.05 M KCl as a background solution.
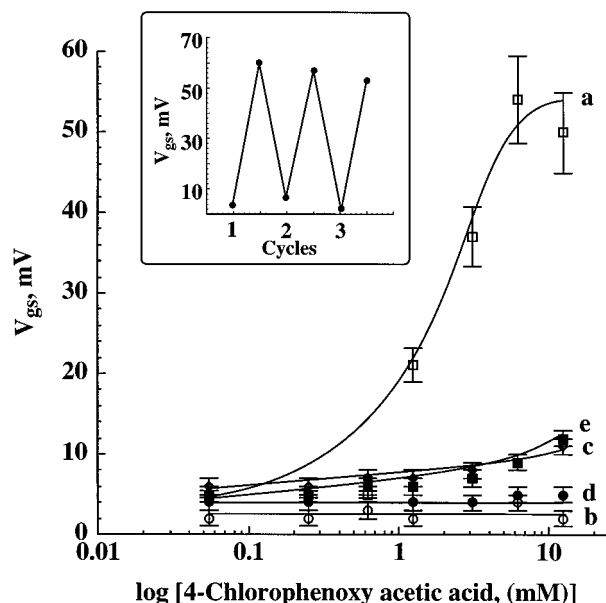
Figure 2. The gate-source voltage, (V_{gs}), of the device upon (a) interaction of the device with the **1**-imprinted TiO_2 film with varying concentrations of Na^+-**1**; (b) interaction of the device, which includes a nonimprinted TiO_2 film on the gate, with varying concentrations of Na^+-**1**; (c) interaction of the **1**-imprinted device with varying concentrations of 2,4-dichlorophenoxy acetate, Na^+-**2**; and (d and e) interaction of the **1**-imprinted device with varying concentrations of cinnamic acid and benzoic acid, respectively. Inset: Cyclic analysis of Na^+-**1** by the **1**-imprinted TiO_2 film-functionalized ISFET device. Upper points correspond to the response of the device after interaction with Na^+-**1** (6.25×10^{-3} M). Lower points correspond to the response of the device after the rinsing of the interface with the bound analyte with ammonium solution, 1% (v/v), followed by rinsing with 0.1 M phosphate buffer solution, pH = 7.2.

Figure 3. The gate-source voltage changes of the ISFET device upon (a) interaction of the **2**-imprinted TiO_2 film sensing interface with varying concentrations of Na^+-**2**, (b) interaction of the device that includes a nonimprinted TiO_2 film on the gate with varying concentrations of Na^+-**2**, (c) treatment of the **2**-imprinted sensing interface with varying concentrations of Na^+-**1**, and (d and e) interaction of the **2**-imprinted sensing interface with varying concentrations of cinnamic acid and benzoic acid, respectively. Inset: Time-dependent development of the gate-source voltage of the TiO_2-functionalized ISFET upon interaction with Na^+-**2** (6.25×10^{-3} M).

titanium(IV) butoxide solution only. The resulting device does not lead to any response upon the addition of Na^+-**1** (Figure 2, b). The imprinted TiO_2 sensing interface reveals impressive selectivity. Addition of Na^+-**2** to the **1**-imprinted sensing interface yields a very low response (5 mV·dec^{-1}) of the ISFET device (Figure 2, curve c), which implies poor interaction and recognition by the TiO_2 film. Similarly, interaction of the **1**-imprinted sensor with cinnamic acid and benzoic acid (Figure 2, curves d, e, respectively) results in low response of the device (3 mV·dec^{-1} and 7 mV·dec^{-1}, respectively).

The possibility for the tailoring of specific chemosensors is further demonstrated by the preparation of an analogous chemosensor for **2** (Figure 1B). Treatment of the resulting film with an ammonia solution, 1% (v/v), yields the tailored imprinted chemosensor for **2**. Figure 3, curve a, shows the change in the gate-source voltage, V_{gs}, of the ISFET upon interaction of the imprinted chemosensor interface with variable concentrations of Na^+-**2**. The lower detection limit of the device is $(1 \pm 0.2) \times 10^{-5}$ M, and the sensitivity of the system for the analysis of Na^+-**2** corresponds to 28 mV·dec^{-1} in the concentration range of 0.1–9.0 mM. The response time of the device, $\tau_{95\%}$, for the sensing of Na^+-**2** is ca. 5 min (Figure 3, inset). The ISFET with the nonimprinted TiO_2 thin film assembled on the SiO_2 gate does not respond to the addition of Na^+-**2** (Figure 3, curve b). The tailored chemosensor reveals selectivity, and interestingly, only a minute output voltage change of the sensor is observed in the presence of 4-chlorophenoxy acetic acid (**1**) (Figure 3, curve c). In addition, the very low
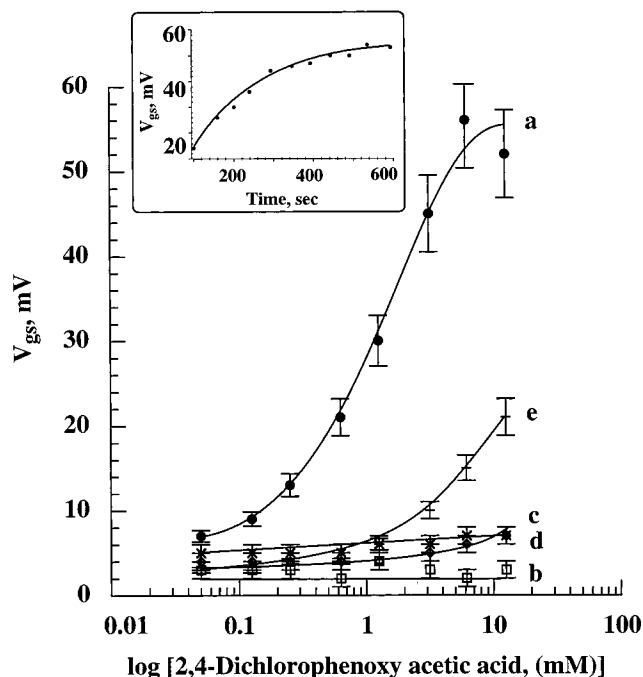
response (4 mV·dec^{-1}) of the device has been observed upon the interaction of the **2**-imprinted interface with cinnamic acid (Figure 3, curve d). It should be noted however that the **2**-imprinted interface is affected by benzoic acid (Figure 3, curve e). Although the detection limit of the device for benzoic acid is substantially lower, the slope of the calibration curve in the region of 10^{-3}–10^{-4} M of benzoic acid corresponds to 18 mV·dec^{-1}.

A further control experiment has involved an attempt to generate similar recognition sites in the presence of **1** and Si-$(OMe)_4$. The resulting SiO_2 interface did not reveal any sensing features for Na^+-**1**. The responses of the TiO_2-functionalized sensors upon the addition of Na^+-**1** or Na^+-**2** may be attributed to a change in the charge that is associated with the TiO_2 film (eq 1). The hydroxytitanate residue that is formed upon the basic hydrolysis of the carboxylate host molecule undergoes deprotonation and the resulting negative charge on the gate interface controls its potential. The binding of the carboxylate analyte generates a titanium ester[17] that depletes the hydroxyl function and the charges on the gate surface. This alters the gate potential and enables the electronic transduction of the respective sensing events. The high selectivity of the sensing interface is attributed to the formation of rigid molecular contours for the respective acid upon the polymerization of the TiO_2 film on the gate interface. The formation of these molecular contours may be facilitated by H-bonds between the polymerizing Ti–OH sites and the carboxy-

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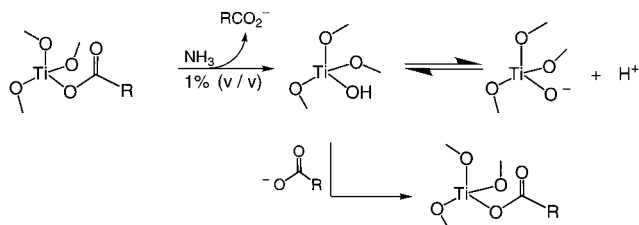
late residues. These imprinted molecular contours result in selective recognition sites for the affinity binding of the imprinted substrate. It should be noted that within the entire concentration ranges for the analysis of Na^+-**1** and Na^+-**2**, the ISFET devices respond almost identically for the respective acid and the Na^+ salt. That is, the buffer capacities of the electrolyte solutions preserve the pH of the media upon the addition of the acids

In order to gain further insight into the nature of the carboxylic acid molecular recognition sites, a TiO_2 film that included $^{14}CH_3$-CO_2H was deposited onto the FET surface. The amount of TiO_2 deposited onto the devices corresponds to 2.6×10^{-2} μmol (9 μg). By following the radioactive-labeled acid that is eliminated from the interface upon interaction with ammonia,[18] the number of imprinted sites associated with the TiO_2 film is estimated to be ca. 1.2×10^{19} sites/g. The resulting TiO_2 film was then subjected to varying bulk concentrations of radioactively labeled acetic acid ($^{14}CH_3CO_2H$). The acid-equilibrated sensing interfaces were subsequently emptied by ammonia to asses the amount of acid

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(18) The fact that, upon sol−gel assembly of the ^{14}C-acetic acid-functionalized TiO_2 film, no free acid is observed when the interface is rinsed indicates that all of the acid that was used in the precursor mixture was incorporated into the resulting TiO_2 film. This suggests that the number of imprinted recognition sites is probably very similar for the different carboxylic acids.

associated with the TiO_2 film at each bulk concentrations of the acid. From these measurements, the association constant of acetic acid to the imprinted sites was estimated to be $K_a = 50 \pm 5$ M^{-1}.

Furthermore, the radioactive measurements indicate that upon removal of the $^{14}CH_3CO_2H$ from the ISFET device, only ca. 70% of the initial radioactivity of the interface is depleted, and ca. 30% of the original radioactivity remains firmly associated with the surface. This suggests that ca. 30% of the original templating acid cannot be removed from the imprinted matrix. The gate interfaces that include the **1** or **2**-imprinted matrixes reveal constant V_{gs} values after interaction with NH_3 for 2 min. Thus, all removable templating compounds are depleted from the immobilized matrixes after this time interval. Although the residual templating carboxylic acids, which are firmly associated with the gate interface, do not interfere with the sensing processes of Na^+-**1** or Na^+-**2**, their possible cooperative contribution to the resulting signal adversely affects the sensitivity and the detection limit of the respective systems.

In conclusion, we have demonstrated a method for the preparation of tailored chemosensors by the imprint of molecular recognition sites for target analytes in TiO_2 thin films associated with the gate interface of ISFET devices.