Chiral Sensors

DOI: 10.1002/ange.200704346

Opposite Signs of Capacitive Microsensor Signals upon Exposure to the Enantiomers of Methyl Propionate Compounds**

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Many biologically active molecules are chiral, and the enantiomeric composition of pharmacologically relevant chiral substances should be known to exclude unwanted side effects.[1–3] A reliable and fast chiral discrimination still represents a major challenge in sensorics because of the identical physicochemical properties (with the exception of chiroptics) of the respective enantiomers in an achiral environment.[4–6] The signals of sensors coated with chiral-recognition structures usually vary only in the magnitude of their response to the different enantiomers.[4–8] Herein, we demonstrate the principle of a capacitive chiral sensor, which provides antipode signals upon exposure to the enantiomers of methyl propionate compounds, so that it is possible to unambiguously determine the enantiomer identity. The dielectric coefficient of the analyte–receptor complex of one enantiomer is significantly different from that of its counterpart. The results disclosed herein show that simple sensor techniques can be used to detect the subtle effects of the different molecular orientations.

Chiral separation techniques, such as electrophoresis and chromatograpy, are widely used and established methods.[9–12] Chiral sensors for the liquid phase (for example, potentiometric sensors)[7,8] have been more extensively studied[4,6,8,13] than the sensors for the gas phase.[5,6] Separation and sensor methods predominantly rely on the use of matrices that contain enantioselective receptor structures,[6,9–16] such as cyclodextrins,[5,6,11,16] or molecularly imprinted polymers.[17,18] The chiral recognition is then based on the formation of a more stable diastereomeric complex with one of the enantiomers. The larger the Gibbs-energy difference between the diastereomeric complexes, the higher the enantioselectivity.

The capacitive transducer used herein forms part of a multitransducer system and features two sets of interdigitated electrodes (see Figure 1), which correspond to two capacitor plates.[20] The sensitive layer consists of chiral octakis(3-*O*-butanoyl-2,6-di-*n*-*O*-pentyl)-γ-cyclodextrin[21] dissolved (50% weight) in a poly(dimethylsiloxane) (PDMS), matrix.[5,6,11] Another sensor chip coated with pure, achiral PDMS is measured simultaneously and acts as a reference. The sensitive layers are thick enough (about 4 μm) so that the volume extension of the electrical field is completely filled, which simplifies the interpretation of the results (because swelling effects can be neglected).[20]

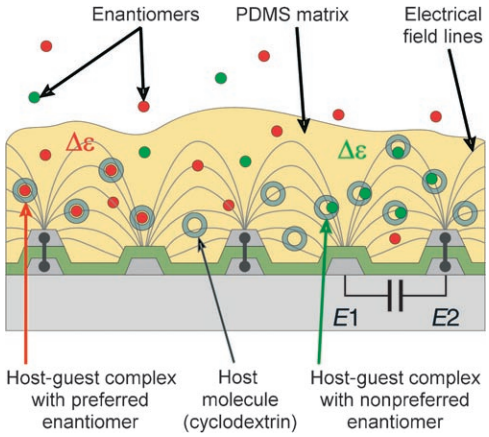

Figure 1. Working principle of the capacitive microtransducer, which includes 128 finger pairs. Electrode width and spacing are 1.6 μm; the measurement frequency is 600 kHz. The capacitance changes upon analyte absorption are in the attoFarad range and cannot be measured conventionally. Therefore, a differential measurement scheme, with a polymer-coated sensing capacitor and a tunable, passivated reference capacitor (silicon nitride passivation layer; no signal is observed upon analyte exposure) was used, in which changes of the minute loading currents of both capacitors upon analyte absorption are measured and compared by using dedicated electronics.[19] The sensor baseline is established by matching the tuneable reference capacitor as closely as possible to the sensing capacitor and then setting the sensor output to zero before starting a measurement. The final output is a frequency signal, which is proportional to the capacitance change upon analyte absorption.

The most important findings are displayed in Figure 2a, which evidences antipode signals upon dosage of the two enantiomers, (*S*)- and (*R*)-methyl-2-chloropropionate, at low analyte concentrations (namely, 20 to 80 ppm). Methyl-2-chloropropionate exhibits a dielectric coefficient of 15. The coefficient of the modified cyclodextrin is 4.0 and that of the cyclodextrin/PDMS mixture is 3.1 (while that of PDMS is 2.7).

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[**] Financial support was provided within the framework of a European Network of Excellence, GOSPEL, FP6-IST-507610, by the Swiss Bundesamt für Bildung und Wissenschaft.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

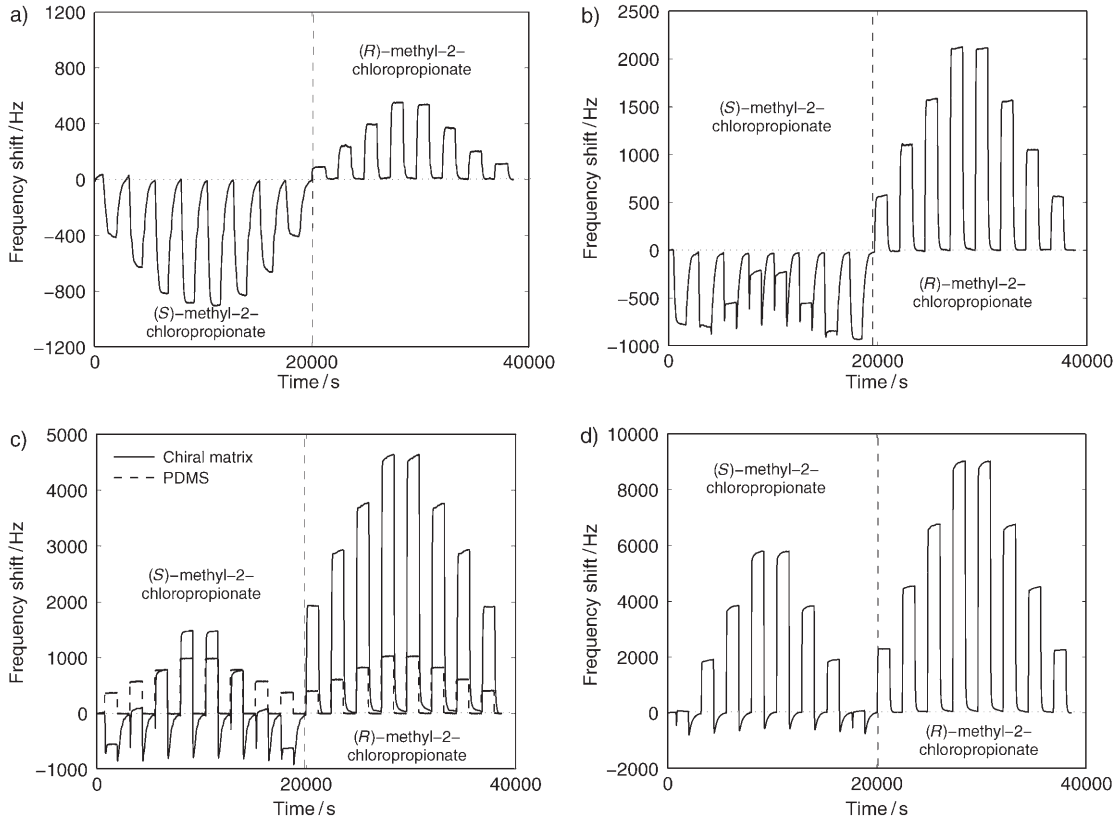

Figure 2. Response of the capacitive sensor upon exposure to the enantiomers of methyl-2-chloropropionate at different concentrations. The measurements are given as frequency shifts, as explained in the caption of Figure 1. For the devices reported herein, 1 Hz corresponds to a capacitance change of 5.25 attoFarad. Analyte concentrations: a) 20, 40, 60, 80 ppm; b) 80, 160, 240, 320 ppm; c) 320, 480, 640, 800 ppm; d) 400, 800, 1200, 1600 ppm. Figure 2c also displays the response of an achiral reference sensor (pure PDMS, dashed line).

The dielectric coefficient of the analyte is larger than that of the sensitive layer, so that a capacitance increase—or a positive sensor signal—should be obtained upon analyte uptake.

The above consideration holds true for the *R* enantiomer (Figure 2a–d), but seems not to be valid for the *S* enantiomer. Since the dipole moments and dielectric coefficients of both enantiomers are the same, any differences observed in their sensor responses must result from a difference in enantiomer–cyclodextrin interactions.

All evidence[5,6,11,21] shows that the *S* enantiomer interacts more strongly with the modified cyclodextrin. We hence ascribe the negative capacitive sensor signal at low analyte concentrations (Figure 2a) to the presence of orientation effects between the (*S*)-methyl-2-chloropropionate molecule and the cyclodextrin, which entail a local compensation of partial charges and/or obstruct molecular reorientation in the low-frequency electric field (600 kHz). These orientation effects significantly decrease the dielectric coefficient of the diastereomeric (*S*)-guest–host complex, thus leading to an overall capacitance decrease. The orientation effect seems to be so strong that any effects of the higher analyte dielectric constant are outbalanced, and the absolute values of the signal are even larger than those of the less-preferred enantiomer at low concentrations (see Figure 2a).

It is striking that only the *S* enantiomer exhibits this orientation effect, whereas the *R* enantiomer behaves as expected—taking into account the dielectric constant of the sorption matrix and the analyte. This observation is even more remarkable, because any interaction can be easily reversed at room temperature by purging with pure air, so that it must be assumed that a favorable superposition of weak interactions causes this effect (instead of strong chemical interactions). According to the literature, the inclusion of the analyte into the cyclodextrin cavity predominantly contributes to enantiomeric discrimination, although interactions may also occur at other sites of the cyclodextrin torus. The host–guest ratio is prevalently 1:1.[4,6,16] Considering that two-dimensional nuclear Overhauser effect spectroscopy (NOESY) is one of the few viable methods to study such orientation effects, we conducted NOESY measurements in our system and found cross-peaks for the *S* enantiomer between the H^{III} atom of the cyclodextrin (which is located inside the cavity, near the wide rim of the cyclodextrin torus) and the methoxy and CH groups of (*S*)-methyl-2-chloropropionate. No nuclear Overhauser effects (NOEs) were detected for other cyclodextrin H atoms located inside the cavity, but further away from the wide rim. In the case of the *R* enantiomer, NOEs were found only between cyclodextrin-H^{III} and the (*R*)-methyl-2-chloropropionate methoxy group,

but not for the CH group. From these data, it can be concluded that both enantiomers enter the cyclodextrin cavity from the wide rim, with the methoxy group being more deeply inserted into the cavity. The data also suggest that the *S* enantiomer penetrates deeper into the cavity than the *R* enantiomer—or that the axis connecting the methoxy oxygen atom of the *S* enantiomer with the carbon atom of the CH group is tilted with respect to the cyclodextrin C_8 axis, so that its CH group comes closer to H^{III} of the cyclodextrin. Either way, the interaction with the *S* enantiomer is stronger, as reflected in the larger NMR-derived association constants [$193 \pm 20\,\text{M}^{-1}$ for *S* and $45 \pm 5\,\text{M}^{-1}$ for *R*]. The involvement of the methoxy group in the chiral recognition process was established earlier in concurrent surface acoustic wave sensor and FTIR measurements.[22]

The signals of the *R* enantiomer increase with rising concentration (see Figure 2a–d), whereas the signals of the *S* enantiomer seem to be difficult to interpret. With increasing analyte concentration, the number of *S* analyte molecules supersedes that of cyclodextrin molecules (1:1 complexation at approximately 100 ppm, see Figure 2b,c). The signal contribution of the orientation effect (that is, the decrease in the dielectric coefficient) is more and more counterbalanced by the dielectric-coefficient increase that originates from analyte molecules for which no cyclodextrin cavities are available; these molecules absorb nonspecifically somewhere else in the cyclodextrin/polymer matrix (see Figure 2b,c). Finally, at large concentrations of the *S* enantiomer, a capacitance increase is observed, which, however, is lower than that produced by the same concentration of the *R* enantiomer (Figure 2d). This result is also conclusive, because a fraction of the *S* analyte molecules is trapped within the cyclodextrin cavities, so that a certain number of nonspecifically absorbed *S* molecules is needed to counterbalance the capacitance-lowering effect caused by the trapped ones. The *R* molecules do not come in close enough contact with the cyclodextrin torus to produce such orientation effects (Figure 2a–d). The response of a PDMS-coated achiral reference sensor was always measured simultaneously (see Figure 2c) to demonstrate that the concentrations of both enantiomers that have been dosed to the sensors were indeed identical.

The plot of the sensor signal versus the analyte concentration is almost linear for the *R* enantiomer (see Figure 3a), which is characteristic for nonspecific physisorption processes (Henry-type sorption). In the case of the *S* enantiomer, the plot exhibits a strongly curved low-concentration part, which goes through the negative region, followed by a linear high-concentration part. The low-concentration part can be best described as a Langmuirian-type curve, because of the limited number of sorption sites inside the cyclodextrin cages, whereas the high-concentration part exhibits the characteristics of a Henry-type sorption.[5] The turning point in the negative region (at 100 ppm) coincides with full receptor coverage (as it was shown in parallel cantilever measurements). These findings were confirmed with methyl lactate using both the same cyclodextrin host (see Figure 3b) and a modified β-cyclodextrin (not shown, see the Supporting Information). It is evident from Figure 3b—which is also

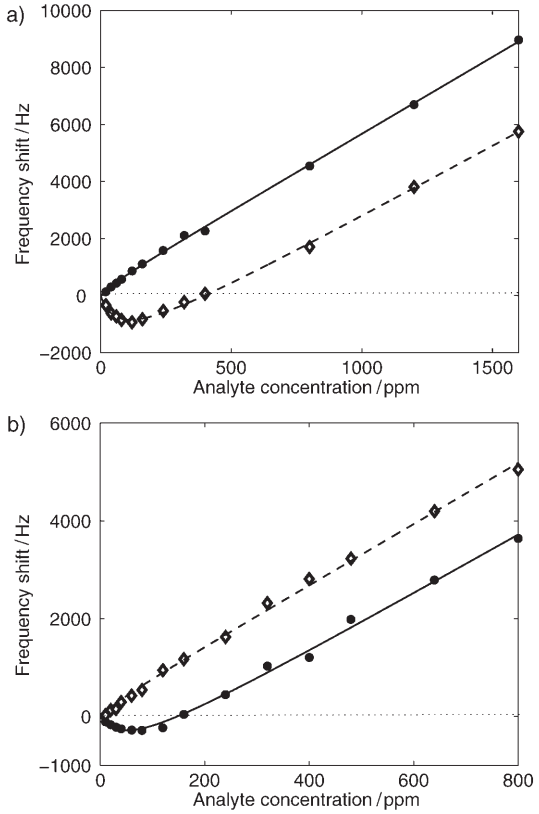

Figure 3. Response of the capacitive sensor as a function of the concentration upon exposure to: a) (*S*) (◇) and (*R*) methyl-2-chloropropionate (●), and b) (*S*) (◇) and (*R*) methyl lactate (●). The lines serve as a guide to the eye.

consistent with literature results[5,11]—that the *R* enantiomer of methyl lactate interacts more intensely with the modified γ-cyclodextrin than the *S* enantiomer, so that the response curves are interchanged with respect to the chloropropionate. Although the dielectric coefficient of methyl lactate is as high as 31, a negative signal was also observed for the preferred enantiomer.

It should be noted that the sensor signals represent a convolution of: 1) the analyte sorption thermodynamics, as these can also be observed in gas chromatography (GC) measurements or with mass-sensitive sensors,[5] and 2) transducer-specific analyte-induced effects on the dielectric properties of the overall layer (molecular orientation effects). A direct comparison of the sensor data with the chiral discrimination factors (α) commonly employed in GC (that is, 4.19 for methyl-2-chloropropionate and 1.54 for methyl-2-lactate, at 303 K) is not possible because of the occurrence of negative and positive signs of the sensor signal. The results show that unequivocal enantiomeric discrimination is only possible at concentrations below the full receptor coverage (see Figure 3), which means that a predilution stage to decrease the overall analyte concentration may be helpful.

In summary, we have shown that our capacitive sensor provides antipodal signals upon exposure to the enantiomers of methyl propionate compounds. The underlying mechanism can be elucidated through spectroscopic measurements.

Sensitive detection of important industrial chemicals, such as pharmaceutical precursors, acidulants, or food additives, in a background composed of other nonchiral standard organic solvents is possible because their enrichment in a cyclodextrin matrix is significantly larger (one order of magnitude).[5] If several chiral analytes with different enantiomeric compositions are present, array strategies using different chiral sensors have to be applied. The findings reported herein can probably be extended to other relevant analytes.

Experimental Section

Octakis(3-*O*-butanoyl-2,6-di-*n*-*O*-pentyl)-γ-cyclodextrin[21] was mixed with PDMS (Silicone GE SE-30, Supelco, Bellefonte, PA, USA) at 50% (w/w), using dichloromethane as solvent, and then deposited on the transducer by means of spray coating. The chiral analytes, that is, both enantiomers of methyl lactate (purity: 98%, Sigma Aldrich AG, Steinheim, Germany) and methyl 2-chloropropionate (purity > 99%, Sigma Aldrich AG, Steinheim, Germany), were dosed to the sensors in a computer-controlled gas manifold, as described in detail elsewhere.[20] Sensors with enantioselective coatings and sensors with achiral polymers (PDMS) were simultaneously measured (for control purposes) in a temperature-regulated flow-through chamber (at 303 K and a sampling frequency of 1.5 Hz, see Figure 2c).[20] Typical experiments consisted of alternating exposures to pure and analyte-loaded air (20 min exposure intervals were used to reach thermodynamic equilibrium).

NMR spectroscopic data were acquired using a Bruker DRX600 spectrometer, equipped with a triple-axis gradient TXI (H/C/N) probe, operating at a field strength of 14.1 T. The resonances of the cyclodextrin derivative were assigned using a 2QF-COSY spectrum (the resonance assignment for methyl-2-chloropropionate is trivial). The 2D-NOESY spectra were recorded at 35 °C, with a mixing time of 250 ms and a spectral resolution of 0.95 Hz pt^{-1} in the direct dimension and 3.80 Hz pt^{-1} in the indirect dimension.

Received: September 20, 2007
Revised: October 15, 2007
Published online: December 20, 2007

Keywords: chirality · cyclodextrins · dielectric properties · electrodes · sensors

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