Molecularly imprinted polymer based extended-gate field-effect transistor chemosensors for phenylalanine enantioselective sensing†

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Chemosensing systems were devised for the enantioselective determination of D- and L-phenylalanine (D- and L-Phe). As recognition units of these systems, molecularly imprinted polymers (MIPs) were designed, guided by DFT calculations, and then synthesized. For the preparation of these MIPs, carboxy derivatized bis(bithiophene) was used as the functional monomer. Both templated and template-extracted MIP films as well as non-imprinted polymer (NIP) films were characterized by IR spectroscopy to prove Phe templation, and then extraction. Extended-gate field-effect transistors (EG-FETs) served as transducers. The EG-FET gates were coated with D- or L-Phe-templated MIP films, by electropolymerization, to result in complete chemosensors. These chemosensors rapidly and selectively responded to D- and L-Phe enantiomer analytes. They readily discriminated between a homologous series of analytes differing by a single atom as well as pairs of enantiomers differing in their three-dimensional structures. Linear dynamic concentration ranges for D- and L-Phe extended from 13 to 100 μM. For both Phe enantiomers, the limit of detection was 13 μM. The enantioselectivity factor was ~2.3 for both chemosensors.

Introduction

Sensing and separating chiral compounds is very important. Most biological compounds and several pharmaceuticals are chiral.† Chiral drugs differ in their pharmacokinetic and pharmacodynamic properties. In many examples, one enantiomer shows the desired effect (like healing properties) while the effect of the other is not desired, e.g., toxicity. For instance, L-DOPA, used in the treatment of Parkinson’s disease, has the u-DOPA enantiomer, which is responsible for the deficiency of white blood cells and, therefore, susceptibility to infections. Similarly, l-methotrexate is better absorbed in the gastrointestinal tract than D-methotrexate, and D-propranolol is more extensively bound to proteins than L-propranolol. Therefore, there is a high demand for the selective recognition of these enantiomers and, thus, separation.

Until now, high-performance liquid chromatography (HPLC) has widely been used for the separation and quantification of chiral isomers. The use of chiral HPLC columns to separate enantiomers is very common. However, more than one chromatographic system is needed to achieve the desired selectivity if multiple chiral isomers are to be separated.

Other than HPLC methods, chemosensing can be an alternative for the selective sensing of enantiomers. The first chemosensing step involves selective interactions of the analyte with the recognition unit immobilized on a transducer surface. This transducer, i.e., an electronic part of the chemosensor, quantifies the effects of the above interactions. Compared with other chemosensors, including electrochemical, acoustic, and optical chemosensors, electric chemosensors based on field-effect transistors (FETs) are favored nowadays because of their advantages including their small size and possibility of further miniaturization,
ease of handling, high sensitivity, capability of using small sample volumes and low cost. Additionally, a FET transducer does not require any special characteristic property of an analyte, as is the case in electrochemical or optical chemosensors.12–16

Importantly, the recognition unit provides the desired selectivity to the chemosensor. Similar to immunology and enzymology, polymer chemistry contributes to the development of new molecular recognition units of high selectivity. Towards that, the application of molecularly imprinted polymers (MIPs) is steadily growing.17–20 Molecular imprinting results in a polymer network wrapped around template molecules. That way, hollow structures complementary in their size, shape, and orientation of recognition sites to the analyte molecules are imprinted in the polymeric matrix. After removal of the template, molecular cavities are generated.21

The analyte recognition properties of MIPs have been demonstrated in an increasing number of examples including those of biological samples.19,29–31 For the latter, MIPs are used as analogues of natural recognition systems.

Template bleeding is a serious drawback of MIPs, if applied as stationary phases in LC columns or SPE cartridges. This leaking during on-line measurement can lead to false results.32,33 In contrast, if an MIP is applied as a recognition unit in a chemosensor, this leaking does not interfere much because the analytical signal is generated only when the molecule binds to its imprinted cavity.

Pioneering aspects of the present research include the implementation of the molecular imprinting strategy for solving problems of the trace level sensing of amino acids. As representative examples, L-Phe and D-Phe were chosen to serve as chiral templates. L-Phe is an essential amino acid. Human body transforms L-Phe into tyrosine and some neurotransmitters or their precursors, such as L-DOPA, adrenalin, and noradrenalin.34 If the body lacks the enzyme responsible for L-Phe transformation, L-Phe is accumulated at a high level. This metabolic disorder is called phenylketonuria. In contrast to L-Phe, the D-Phe enantiomer is not found in food. Importantly, only the D-Phe enantiomer was proposed out of the two enantiomers as a chronic pain reliever.35

Some animal studies confirmed the D-Phe use to cure depression associated with Parkinson’s disease.

Various efforts were undertaken to imprint Phe (Table 1).23–28 However, most of the reported MIP chemosensors for Phe could not discriminate between the Phe enantiomeric forms.23,25,26,36

Moreover, selectivity and sensitivity of these chemosensors were not appreciable. Additionally, most of prepared MIPs were devised to serve as separation materials. In contrast, the present work proposes an easy and effective way of preparation of MIP films and their use as recognition units, integrated with extended-gate field-effect transistor (EG-FET) transducers, to devise chemosensors for the highly selective and sensitive determination of L- and D-Phe. Selective molecular cavities were generated in MIPs by using carboxy derivatized bis(bithienyl)methane as the functional monomer. The EG-FET gate surface, coated with either the (D- or L-Phe)-templated MIP film, rapidly and selectively responded to D- or L-Phe enantiomer analytes. Advantageously, these chemosensors discriminated between a homologous series of analytes differing by a single atom and enantiomers differing only in their three-dimensional structure.

### Experimental

#### Reagents

Synthetic details of the preparation of the functional monomer, p-bis(2,2’-bithienyl-5-yl)benzoic acid 3, and the cross-linking monomer, 2,4,5,2’-tetra(hthiophen-2-yl)-3,3’-bithiophene 4, used in the present work are described elsewhere.37–40 All the synthesized monomers were purified by HPLC before use. Acetonitrile, 2,2’-bithiophene-5-carboxylic acid 7, L- and D-Phe, and other amino acids were purchased from Sigma-Aldrich. Electrochemical grade tetrabutylammonium perchlorate, (TBA)ClO4, was from Fluka. The isopropanol solvent and phosphate salts were procured from CHEMPUR.

#### Instrumentation and procedures

An AUTOLAB computerized electrochemistry system of Eco Chemie, equipped with the expansion cards of the PGSTAT 12 potentiostat and the FRA2 frequency response analyzer controlled by the GEPES 4.9 software of the same manufacturer, was used for deposition of thin polymer films. Piezoelectric microgravimetry (PM) measurements were carried out with a Model 5710 electrochemical quartz crystal microbalance (EQCM 5710) of IPC PAS. The resonance frequency change was measured with 1 Hz resolution using a 14 mm diameter, AT-cut, plano–plano quartz.

### Table 1 MIP based analytical methods developed for the determination of phenylalanine

<table>
<thead>
<tr>
<th>Functional monomer/cross-linking monomer/porogen</th>
<th>Form of MIP prepared</th>
<th>MIP application</th>
<th>Enantiomer separation</th>
<th>Analytical parameter</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methacrylic acid/EGDMA/toluene</td>
<td>Bead (diameter 28 μm)</td>
<td>Separation</td>
<td>Yes</td>
<td>Low enantioselectivity (1.28)</td>
<td>22</td>
</tr>
<tr>
<td>Methacrylic acid/EGDMA/ACN</td>
<td>Bulk</td>
<td>Separation</td>
<td>No</td>
<td>Phe separation from other amino acids</td>
<td>23</td>
</tr>
<tr>
<td>Acrylonitrile/acrylic acid/DMSO</td>
<td>Film</td>
<td>PM sensor</td>
<td>No</td>
<td>LOQ = 50–500 mg L⁻¹, LOD = 45 mg L⁻¹</td>
<td>24</td>
</tr>
<tr>
<td>Poly styrene-co-polyethylene</td>
<td>Film</td>
<td>SPR sensor</td>
<td>No</td>
<td>LOD = 0.5 mM</td>
<td>25</td>
</tr>
<tr>
<td>Acrylamide/EGDMA/ethanol</td>
<td>Bead (diameter ~ 0.2 to 1 μm)</td>
<td>Optical sensor</td>
<td>No</td>
<td>LOQ = 1.3 μM–0.5 mM, LOD = 0.6 μM</td>
<td>26</td>
</tr>
<tr>
<td>β-Cyclodextrin derivatized vinyl carboxylic acid/N,N’-methylene bisacrylamide/water</td>
<td>Polymer prepared in spaces between beads of self-assembled poly styrene colloidal crystal</td>
<td>Optical sensor</td>
<td>Yes</td>
<td>10 nM–0.1 mM, LOD = 0.6 nM</td>
<td>27</td>
</tr>
<tr>
<td>Poly(ethylene-co-vinyl alcohol)</td>
<td>Core–shell bead (100–300 nm diameter) with a magnetic core</td>
<td>Separation</td>
<td>No</td>
<td>Binding to catecholamine hormones; the isotherm study from ~25 to 100 μg mL⁻¹</td>
<td>28</td>
</tr>
</tbody>
</table>

ACN, acetonitrile; DMSO, dimethylsulfoxide; EGDMA, ethylene glycol dimethacrylate; LOD, limit of detection; LOQ, limit of quantification; PM, piezoelectric microgravimetry; SPR, surface plasmon resonance.
crystal resonators (Au-QCRs) of 10 MHz resonance frequency with 5 mm diameter and 100 nm thick Au film electrodes over a Ti underlayer on both sides. For electropolymerization, which was performed under stagnant-solution conditions, EQCM 571041 was interfaced with the EP-20 potentiostat of IPC PAS and its quartz crystal holder was mounted horizontally with the Au-QCR facing upward in order to use as low volume of the sample solution as 100 µL. For PM measurements under flow injection analysis (FIA) conditions, a flow-through EQCM 5610 holder of IPC PAS was used. 10 mM NaH₂PO₄ was used as the carrier solution pumped at flow rate of 35 µL min⁻¹.

The structure of the pre-polymerization complex was computationally modeled using the density functional theory (DFT) at the B3LYP level with the 3-21G* basis set, all implemented in the Gaussian 2009 software package. For determining the average film thickness, some parts of the film were carefully removed in few layers of gold evaporated on titanium underlayers (Au-glass slides). The experimental IR spectra were compared with those deposited from the template-free solution using the DFT method within harmonic approximation. The positions of the bands in experimental spectra were determined using the procedure implemented in the Gaussian 2009 software package. Calculated normal modes were assigned to experimental bands in two steps. The first Vibrational Energy Distribution Analysis (VEDA) step calculated normal modes expressed in terms of local modes (vibrations of internal bonds, bond angles, and dihedral angles). In the second step, the spectra calculated were fitted to experimental data by means of linear regression. That way, the unharmonic factor was phenomenologically introduced to calculate frequencies. Those frequencies were scaled with the SPESCA program.

Polymer surfaces were imaged by atomic force microscopy (AFM) using a multimode 8 microscope equipped with the Nanoscope V controller, both from Bruker. ScanAsyst Mode imaging was performed with a silicon tip mounted on a nitride cantilever of 70 kHz resonance frequency. For this imaging, the polymer films were deposited on the (7 x 4) mm² strips of the Au film coated glass slides. For determining the average film thickness, some parts of the film were carefully removed in few different places from the electrode surface, i.e., scratched with a Teflon™ spatula, under an optical microscope. Subsequently, these scratches were imaged using AFM. Then, heights of the resulting steps were measured by averaging the number of points on both sides of the step (sufficiently far from its partially detached front). The difference between the average values of points on the step and at its foot determined the height of the step. Finally, step heights measured for different

The desired selectivity of MIPs is obtained by suitable design of their molecular cavities. In these cavities, different recognition sites are fixed in a well-defined manner to provide a definite chemical microenvironment for reversible binding of target analyte molecules. Selection of functional monomers with recognition functionalities complementary to analyte binding sites is very important for the formation of a stable pre-polymerization complex, hence ultimately resulting in well-defined molecular cavities in the polymer matrix. Formation of this complex in solution is often optimized by quantum chemistry calculations. Advantageously, the software development along with the increase of the power of computing made
molecular simulations exclusively based on mathematical modeling feasible.

The change in the Gibbs free energy of formation of the complex, $\Delta G$, of $\alpha$- or $\delta$-Phe with different functional monomers was calculated using the density functional theory (DFT) method. The higher the negative change of this energy, the higher is the stability of the pre-polymerization complex formed. Several different functional monomers with different recognizing functionalities were tested for finding the most suitable one. Scheme S1 (ESI†) shows the structural formulas of four examples out of the functional monomers used for computer modeling. Table S1 (ESI†) summarizes the $\Delta G$ values calculated for the formation of pre-polymerization complexes with these monomers. With this preliminary screening step, the most stable complex-forming functional monomer $3$ was selected.

Scheme 1 shows the structural formula of the pre-polymerization complex revealing possible multipoint interactions between two molecules of functional monomer $3$ and the $\alpha$-Phe template in vacuum. In the optimized complex (Scheme 1b), hydrogen atom $39$ of $3'$ forms a hydrogen bond with the nitrogen atom $1$ of $2$. Similarly, the hydrogen atom $16$ of $3$ interacts with the oxygen atom $3$ of $2$. Moreover, the oxygen atom $2$ of $3$ forms a hydrogen bond with the hydrogen atom $27$ of $2$. These multipoint interactions stabilize the complex. The calculated value of $\Delta G$ was remarkable, i.e., it equaled $-119$ kJ mol$^{-1}$ (in vacuum). Interestingly, an even higher negative $\Delta G$ value ($-147.2$ kJ mol$^{-1}$) was obtained when we considered the medium (ACN) effect on this complexation. Apparently, the increase of medium polarity favored the formation of a pre-polymerization complex in solution. Moreover, we considered the medium effect on the dimerization of the template molecules and functional monomer molecules (Table S1, ESI†). It appeared, however, that changes in $\Delta G$ for both dimerizations were lower than that for pre-polymerization complex formation.

Fig. 1 shows the current–potential curves for the potential-dynamic electropolymerization of $\alpha$-Phe (Fig. 1a) and $\delta$-Phe (Fig. 1b) resulting in the deposition of respective MIP films on the Pt disk electrodes. In the first cycle, the anodic peak at $\sim1.0$ V corresponds to the irreversible electro-oxidation of the $\text{bis}[\text{bithiophene}]$ moiety. During this electro-oxidation, a radical cation is formed. This peak decreased in subsequent cycles indicating the formation of a less conducting MIP film.

After rinsing with acetonitrile, the $\alpha$- or $\delta$-Phe template was extracted from the MIP film for the application of this film as the chemosensor recognition unit. To control the progress of Phe removal, the gate effect of the MIP film was examined (Fig. 2). For that, the electro-oxidation of a $\text{K}_{3}\text{Fe(CN)}_{6}$ redox probe was followed by DPV at the MIP film coated electrode for different times spans of template extraction. Apparently, removal of the template from the MIP molecular cavities resulted in the increase of the DPV peak current for the redox probe oxidation (Fig. 2). Finally, $90$ min extraction with $10$ mM NaOH appeared to be optimized (curve 5 in Fig. 2).

$\alpha$-Phe template extraction from the MIP film was confirmed by the PM IRRAS spectroscopy measurements. Fig. S1 (ESI†) presents the experimental spectra recorded for the MIP film before and after $\alpha$-Phe extraction and the NIP film along with calculated and scaled theoretical frequencies of normal modes. The band assigned to the perchlorate anion was removed out of the functional monomers used for computer modeling.

The morphology of the polymer plays an important role if it is used as a recognition unit of a chemosensor. The polycrystalline morphology is preferable because a polycrystalline organic polymer film exhibits better sensing properties than an amorphous polymer film in terms of response, repeatability, and reproducibility. Therefore, the morphology of polymer films was herein examined with AFM imaging.

A distinct surface morphological pattern was observed in AFM images of different MIP and NIP samples (Fig. 3). Brighter areas
in the images correspond to bigger polymer grains. Interestingly, the (D-Phe)-templated MIP film was enriched with grains of bigger size, ~70 nm (Fig. 3a). However, smaller grains, ~35 nm, appeared (Fig. 3b) after the extraction of the template with 10 mM NaOH. The morphology of the NIP film was similar to that of the (D-Phe)-templated MIP film (Fig. 3c), i.e., it was composed of bigger grains, ~69 nm. Moreover, the thickness of the MIP film before the extraction of the D-Phe template equaling 224 ± 14 nm decreased to 175 ± 51 nm after extraction. Presumably, this decrease resulted from the removal of loose parts of the deposited MIP film. The thickness of the NIP film was 221 ± 24 nm.

The morphologies of both MIP and NIP films were further investigated by SEM. The SEM images of the (D-Phe)-templated MIP film before and after template extraction along with those of the NIP film before and after similar treatment are shown in Fig. S2 (ESI†). The imaging showed that the MIP film was composed of small densely packed grains of the same size (Fig. S2a and b, ESI†). However, some irregular granules are also seen on top of smaller grains. NIP films reveal similar morphology (Fig. S2c and d, ESI†). Typically, the conductivity of such a compact film is high,51 a desired property for a FET based chemosensor.

After template extraction, D- or L-Phe analyte binding by respective MIPs was examined. For the determination of this binding, electrical transduction using an EG-FET was applied. In comparison to the commonly available metal oxide based FET sensing systems, conducting polymer film based recognition
units are preferred because they can operate at ambient temperature. Moreover, conducting polymers, due to their semiconducting and redox properties, undergo changes in their electrical conductivity upon changes in the number of charge carriers incurred by doping/dedoping, interactions of ions, or charge transfer between molecules. Besides, conducting polymers with higher conductivity may help to overcome problems with Debye length limitations inherent to FET transducers. All of these make the use of conducting polymers in FETs for sensing applications very promising.49

An EG-FET is a MOSFET modification, in which the gate is extended outside of the transistor. Therefore, the operation principle of the EG-FET remains within the scope of the basic MOSFET description. Scheme 2 shows the experimental setup with the MIP film deposited on the working electrode (WE), i.e. the extended gate, and the Pt plate reference electrode (RE). When a positive with respect to the source of the n-type channel MOSFET voltage is applied to the gate, electrons (majority carriers in the substrate) are attracted to the surface of the gate and form a conducting channel between the source and the drain. Fundamentals of the electric method used are described elsewhere.52,53 Accordingly, the transistor characteristics were determined at the constant gate voltage vs. the Pt reference electrode, $V_R = 1.50 \text{ V}$ while the drain voltage ($V_D$) was scanned from 0 to 5.0 V. The change in the resulting drain current ($I_D$) was measured. This change, recorded for different concentrations of the analyte, was used to monitor the binding of the analyte molecule to its molecular cavity from 0 to 5.0 V. The change in the resulting drain current ($I_D$) was attributed elsewhere.52,53

Fig. 4 shows the dependence of the change in the drain current on the $\sigma$- or $\iota$-Phe concentration, as determined from the EG-FET characteristics. The recorded $I_D$ changes are attributed to the interfacial potential shift at the (extended gate)-solution interface. The correlation between the change in the drain current and the analyte concentration in solution was linear up to 100 $\mu$M Phe (both $\sigma$- and $\iota$-Phe form). Importantly, these chiral MIP films were cross-selective. To determine enantioselectivity in both cases, we compared the slopes of calibration plots for $\sigma$- and $\iota$-Phe at MIP-$\sigma$-Phe and MIP-$\iota$-Phe. The MIP-$\sigma$-Phe was highly sensitive to $\sigma$-Phe (curve 1 in Fig. 4a). However, advantageously, the response of this MIP to the addition of $\iota$-Phe was appreciably low (curve 2 in Fig. 4a), which led to the enantioselectivity factor of $\approx 2.3$, determined as the ratio of these slopes. Similarly, the MIP-$\iota$-Phe was very sensitive to $\iota$-Phe and its sensitivity to $\sigma$-Phe (curves 1' and 2', respectively, in Fig. 4b) was low, resulting in the enantioselectivity factor of $\approx 2.3$. This difference in behavior most likely arises from the presence of chiral cavities generated during respective imprinting. Table 2 summarizes the analytical parameters determined for the devised chemosensors.

Fig. 4 Calibration plots for (a) the $\sigma$-phenylalanine)-templated MIP film for (1) $\sigma$-phenylalanine, (2) $\iota$-phenylalanine, and (b) the $\iota$-phenylalanine)-templated MIP film, for (1') $\iota$-phenylalanine (2') $\sigma$-phenylalanine. MIP films were deposited on the Au-glass slide gates of the EG-FET transducer. Curves (3) and (3') are calibration plots for $\sigma$- and $\iota$-phenylalanine, respectively, for the NIP film. $V_R = 1.50 \text{ V}$. 

In order to confirm the imprinting, an NIP film deposited on the Au-glass slide electrode was assembled in the EG-FET system as a control. Because of the absence of molecular cavities, binding of $\sigma$-Phe to the NIP was low (curve 3 in Fig. 4a). Therefore, sensitivity of the NIP electrode towards $\sigma$-Phe was much lower than that of the MIP electrode (Table 2). The chemosensor behaved similarly to $\iota$-Phe (curve 3' in Fig. 4b). Evidently, the low sensitivity of the NIP film to Phe confirmed the importance of imprinting molecular cavities in the MIP. From the ratio of the sensitivity of the MIP-$\sigma$-Phe and NIP chemosensor to $\sigma$-Phe (curves 1 and 3 in Fig. 4a, respectively), the apparent imprinting factor (AIF) was determined to be very high equaling 36.

Then, the selectivity of both MIP films with respect to functionally and structurally similar interferences including $\sigma$-tyrosine, $\sigma$-proline, and $\sigma$-alanine was evaluated (Fig. S3 and S4, ESI†). The results of selectivity studies are summarized in

Scheme 2 The experimental setup, based on the EG-FET design, using an Au-glass slide coated with the phenylalanine-templated MIP film or NIP film as the gate (working electrode, WE) and a Pt plate as the reference electrode (RE). Symbols D and S stand for the drain and source components of the EG-FET structure, respectively. The reference electrode was polarized to $V_R = 1.50 \text{ V}$ (adapted from ref. 50).
In the field of molecular imprinting, the imprinting factor is a measure of the ratio of slopes of calibration curves for the MIP and NIP chemosensors. This factor confirms the suitability of the fabricated chemosensor for selective binding of the analyte to its MIP molecular cavities.

Moreover, the selectivity of the devised chemosensor in complex samples was studied (Fig. 5). For that, the concentration of D-Phe was determined in the presence of equimolar concentrations of L-Phe, D-alanine, L-tyrosine, and D-proline. The results showed only a slight increase of the drain current (~10%) in the presence of these interferences (Fig. 5E), thus confirming the suitability of the fabricated chemosensor for real applications.

**Determination of the imprinting factor**

In the field of molecular imprinting, the imprinting factor is the measure of the concentration of selective molecular cavities in the MIP network. The analytical signal response of the MIP is compared with that of NIP to determine this factor. The strength of multiple interactions of the analyte molecules with matching molecular cavities determines this value. In the present study, a high value of AIF was determined from the ratio of slopes of calibration curves for the MIP and NIP EG-FET chemosensors. However, this approach does not provide the true imprinting factor value. This is because several factors other than the presence of the analyte in MIP, such as the charge or the dipole moment of the analyte, may govern the change in the drain current. Therefore, PM transduction was used to determine true amounts of the analyte bound to the MIP and NIP films.

For that, the MIP-(D-Phe) thin film was deposited on an Au-QCR mounted in the FIA holder. 100 µL sample solutions of increasing D-Phe concentrations were injected under FIA conditions for constructing calibration plots. The change in the resonance frequency (Δf) is opposite to the change in the mass of the Au-QCR with the deposited MIP or NIP film, as the Sauerbrey equation predicts. Each consecutive injection of different concentrations of D-Phe resulted in different changes in resonance frequency, thus confirming D-Phe binding to molecular cavities. Importantly, a similar change in the NIP film coated Au-QCR was much lower.

The measured frequency decrease obeyed the linear regression equation of \( \Delta f \) (Hz) = −7.04 (Hz mM⁻¹) c (mM) − 2.02 (Hz) for the analyte concentration up to 20 mM with a sensitivity of −7.04 ± 0.97 Hz mM⁻¹ and the correlation coefficient of 0.93 (curve 1 in the inset to Fig. 6). Similarly, the resonance frequency changes corresponding to analyte binding by the NIP film were measured to construct the calibration plot (curve 2 in the inset to Fig. 6). The sensitivity of the NIP film to D-Phe (−0.57 ± 0.16 Hz mM⁻¹) was determined from the slope of a similar FIA calibration plot described using the following equation.

\[
\Delta f \text{ (Hz)} = -0.57 (\text{Hz mM}^{-1}) \cdot c \text{ (mM)} - 6.65 \text{ (Hz)}.
\]

The correlation coefficient was 0.74. From the ratio of sensitivity to D-Phe of the MIP-(D-Phe) and that of the NIP, the imprinting factor was determined to be relatively high equaling 12. This relatively high imprinting factor confirms the high concentration of molecular cavities in the MIP. Moreover, this result confirms the superiority of EG-FET transducers over piezoelectric microgravimetry transducers.
Enantiomeric phenylalanine (D-Phe) was selectively detected using MIPs fabricated by electropolymerization on Au-QCR surfaces. The resonance frequency change with time after injection of D-Phe at various concentrations showed a clear detection limit of 13 mM, evidenced by the distinct frequency shifts in MIP and NIP films. The enantioselectivity factor was calculated to be greater than that of the latter (up to 5 mM). These results demonstrate the potential of MIP-based sensors for the selective detection of D-amino acids.

**Conclusions**

We fabricated two EG-FET based MIP chemosensors, each for the enantiomeric phenylalanine (D-Phe). For this determination, the MIP films were deposited by electropolymerization on the surfaces of extended gates of field-effect transistors. The resulting MIPs selectively discriminated between the D- and L-form of phenylalanine and they were selective for D-Phe with respect to the interferences from common amino acids including D-proline, D-alanine, and D-tyrosine.

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